Studies on the developmental and antidepressant-like effects of anesthetics

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”I know, my dear Watson, that you share my love of all that is bizarre and outside the conventions and humdrum routine of daily life”

- Sherlock Holmes -


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Abstract

Anesthetics are commonly used to induce unconsciousness and insensateness during surgery. However, the impacts of anesthetics on brain function go well beyond their acute pharmacological effects. Animal research suggests that the developing brain is particularly vulnerable to anesthesia, and even a single exposure may induce persistent neurobiological and behavioral consequences. Nevertheless, anesthetics have demonstrated remarkable therapeutic potential against some prevalent and debilitating brain disorders, especially major depression. Indeed, a single subanesthetic dose of ketamine has been reproducibly shown to alleviate depression and suicidal thinking within hours of administration, and the effects can last for days. Induction of brain-derived neurotrophic factor (BDNF) receptor TrkB signaling and synaptic plasticity have been intimately connected with ketamine's antidepressant effects, but the precise mechanistic basis remains obscure. Notably, rapid antidepressant effects have also been reported with other anesthetics, including nitrous oxide (N_2O) and isoflurane, and after somatic treatments such as electroconvulsive therapy (ECT) and sleep deprivation.

In the first part of this thesis, we investigated the long-term behavioral effects of early postnatal exposure to repeated brief isoflurane anesthesia. We exposed mouse pups to anesthesia on three consecutive days at two distinct developmental stages, at postnatal days 7–9 or 15–17, and later tested the behavioral phenotype of the adult animals. Isoflurane anesthesia caused modest behavioral effects on locomotor activity and spatial learning and memory of the adult mice, depending on the age of the animals during the anesthesia exposures.

In the second part, we investigated the effects of various anesthetics on depressive-like behavior and molecular signatures connected to the antidepressant effects of ketamine in rodents. We subjected rats to the chronic mild stress model of depression and subsequently exposed them to repeated brief isoflurane anesthesia for a total five times every three days. This administration regimen, however, was insufficient to normalize anhedonic behavior in the stressed rats. Cortical and hippocampal BDNF levels in these rats also remained unaltered.

We then investigated the dose-dependent and temporal effects of different anesthetics on TrkB signaling, activity-dependent immediate-early genes (IEGs), and electroencephalographic (EEG) activity in mice. Here, we discovered that N_2O upregulated several IEGs (markers of cortical excitation) during acute pharmacological effects, and that these effects were followed by a rebound emergence of EEG slow-wave activity (SWA) and TrkB signaling after treatment cessation. Similar concurrent upregulation of SWA and TrkB signaling was evident after a flurothyl-induced seizure (reminiscent of ECT) and during the effects of a sedative drug, medetomidine. Medetomidine, however, lacked antidepressant-like effects in the learned helplessness model of depression. This suggested that instead of only an increase in SWA and TrkB signaling, a preceding excitatory effect is also crucial for rapid antidepressant effects. This may also explain our observed lack of behavioral effects of deep isoflurane anesthesia. Moreover, even though ketamine's antidepressant effects are associated

with subanesthetic doses, we found that ketamine increases SWA and TrkB signaling, with the most pronounced effects observed at high anesthetic-sedative doses. These effects appear independent of hydroxynorketamine, an active metabolite of ketamine that has demonstrated antidepressant-like effects in rodents.

In conclusion, we found subanesthetic ketamine, N_2O, and flurothyl to induce SWA after their acute pharmacological effects subsided. Interestingly, this phenomenon resembles the well-known postictal (i.e., after seizure) slowing of EEG activity, which has been connected to the antidepressant effects of ECT. Furthermore, the emergence of SWA coincided with the upregulation of TrkB signaling. Based on our results, we propose that rapid-acting antidepressants induce two distinct phases in the brain, with an initial excitatory phase followed by a sedative-like brain state that is characterized by SWA and TrkB signaling. Further studies are needed to elucidate whether a similar phenomenon is shared by other treatments that have demonstrated rapid antidepressant effects (e.g., isoflurane, psilocybin). The current proposal provides a novel framework for future research that encourages expanding the research focus from the acute pharmacology of the treatments to homeostatic alterations that potentially emerge as an intrinsic response of the brain to a drug challenge.

Tiivistelmä

Anesteetit ovat pitkään olleet merkittävä osa kliinistä lääketiedettä, mutta ymmärryksemme niiden pitkäaikaisvaikutuksista on yhä puutteellista. Erityistä huolta ovat herättäneet varhaiskehityksen aikaista anestesiaa käsitelleet eläintutkimukset, joissa jo yksittäisen altistuksen on näytetty aiheuttavan pitkäkestoisia neurobiologisia ja käyttäytymistason muutoksia. Toisaalta, anesteeteilla on myös havaittu terapeuttista potentiaalia psykiatristen sairauksien, erityisesti masennuksen hoidossa aikuisilla. Eniten huomiota on saanut ketamiini, jonka on osoitettu lievittävän masennusoireita ja itsetuhoista ajattelua jo tuntien sisään yksittäisestä subanesteettisesta annostelusta, vaikutusten kestäessä parhaillaan yli viikon. Näiden vaikutusten neurobiologinen tausta on yhä osin epäselvää, mutta niiden ajatellaan liittyvän aivoperäisen hermokasvutekijän (BDNF) TrkB-reseptorin välittämiin aivojen muovautuvuuden muutoksiin. Myös muiden anesteettien, kuten isofluraanin ja ilokaasun, sekä somaattisten hoitojen kuten sähköhoidon ja unideprivaation on osoitettu lievittävän masennusoireita nopeasti.

Tämän väitöskirjan ensimmäisessä osiossa tutkimme varhaisen isofluraanialtistuksen pitkäkestoisia käyttäytymisvaikutuksia altistamalla hiiriä kolmelle päivittäiselle isofluraanianestesialle kahdessa eri aikapisteessä varhaiskehityksen aikana, 7–9 tai 15–17 päivän ikäisinä. Myöhemmin testasimme eläinten käyttäytymisfenotyyppiä aikuisiällä. Varhaiskehityksen aikainen toistettu isofluraanialtistus aiheutti lieviä muutoksia aikuisten hiirten lokomotorisessa aktiivisuudessa sekä heikennyksiä muistia ja oppimista mittaavissa testeissä. Vaikutukset riippuivat kehityksellisestä aikapisteestä, jona hiiret altistettiin anestesialle.

Toisessa osiossa tutkimme anesteettien masennuslääkevaikutuksia sekä niiden aiheuttamia muutoksia ketamiinin masennuslääkevasteeseen liitettyihin neurobiologisiin tekijöihin aikuisilla jyrsijöillä. Ensin testasimme toistetun syvän isofluraanianestesian kykyä normalisoida kroonisen lievän stressimallin aiheuttamia käyttäytymismuutoksia rotilla. Viidesti toistettu isofluraanianestesia joka toinen päivä ei kuitenkaan lievittänyt masennuksen kaltaista käyttäytymistä stressille altistetuissa rotissa. Isofluraanilla ei myöskään ollut vaikutusta rottien etuaivokuoren tai hippokampuksen BDNF-pitoisuuteen. Seuraavaksi tutkimme anesteettien annosriippuvaisia vaikutuksia hiirten aivojen sähköiseen aktiivisuuteen elektroenkefalografian (EEG) avulla sekä TrkB:n välittämään solusignalointiin ja aivokuoren eksitaatiomarkkereihin annostelun eri vaiheissa. Havaitsimme ilokaasun lisäävän aivokuoren eksitaatiota välittömien farmakologisten vaikutusten aikana, kun taas annostelun lopettamisen jälkeen havaitsimme EEG:ssä hidasaalto-oskillaatioiden asteittaisen lisääntymisen, jonka aikana myös TrkB-signalointi aktivoitui. Havaitsimme samankaltaisen hidasaaltoaktiivisuuden ja TrkB-signaloinnin yhteisen esiintymisen myös flurotyylillä aiheutettujen kouristusten jälkeen (lääkkeellinen malli sähköhoidosta) sekä sedatiivisen lääkeaine medetomidiinin vaikutusten aikana. Medetomidiini ei kuitenkaan lievittänyt masennuksen kaltaista käytöstä opittu avuttomuus -hiirimallissa. Ketamiini lisäsi hidasaaltoaktiivisuutta ja TrkB-signalointia annosriippuvaisesti siten, että näiden esiintyvyys

oli voimakkainta anesteettisella annoksella, vaikka ketamiinin masennuslääkevaikutus ilmenee tyypillisesti subanesteettisella annoksella. Nämä vaikutukset olivat riippumattomia hydroksinorketamiinista – ketamiinin aktiivisesta metaboliitista, jolla on myös havaittu masennuslääkevaikutusta eläinkokeissa.

Tuloksemme viittaavat siihen, että nopeavaikutteisten masennuslääkkeiden aikaansaamalla akuutilla eksitaatiolla ja sitä seuraavalla hidasoskillaatiotilalla on tärkeä merkitys niiden terapeuttisen vasteen kannalta. Tämä kaksivaiheinen ilmiö muistuttaa sähköhoidon jälkeen havaittavaa EEG-aktiivisuuden hidastumista, jonka voimakkuus on yhdistetty hoidon terapeuttiseen vasteeseen. Tätä taustaa vasten jatkotutkimuksissa tulisikin selvittää, tapahtuuko samanlainen ilmiö myös muiden nopeavaikutteista masennushoitovastetta osoittaneiden käsittelyjen, kuten isofluraanin ja psilosybiinin yhteydessä. Tuloksemme tarjoavat nopeavaikutteisten masennuslääkkeiden mekanistiseen tutkimukseen uuden lähestymiskulman, joka kannustaa tarkastelemaan hoitojen vaikutuksia niiden akuuttia farmakologiaa laajemmin myös lääkkeen eliminaation jälkeen ilmenevien aivojen homeostaattisten muutosten kautta.

Abbreviations

AKT = protein kinase B
AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
BDNF = brain-derived neurotrophic factor
CaMKIV = calcium/calmodulin-dependent protein kinase IV
CMS = chronic mild stress
CNS = central nervous system
CREB = cyclic AMP response element binding protein
DAG = diacylglycerol
ECS = electroconvulsive shock
ECT = electroconvulsive therapy
eEF2 = eukaryotic elongation factor 2
EEG = electroencephalography
ELISA = enzyme-linked immunosorbent assay
EPM = elevated plus-maze
FST = forced swim test
GABA = gamma-aminobutyric acid
GSK3β = glycogen synthase kinase 3β
HC = hippocampus
HNK = hydroxynorketamine
IEG = immediate-early gene
IP_3 = inositol trisphosphate
LSD = lysergic acid diethylamide
LTP = long-term potentiation
MAOI = monoamine oxidase inhibitor
MAPK = mitogen-activated protein kinase; ERK
MDD = major depressive disorder
mGluR2 = metabotropic glutamate receptor subtype 2
mTOR = mammalian target of rapamycin
N_2O = nitrous oxide
NBQX = 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione
NMDAR = N-methyl-D-aspartate receptor
NREM = non-rapid eye movement
PCR = polymerase chain reaction
PFC = prefrontal cortex
PI3K = phosphatidylinositol kinase 3
PKC = protein kinase C
PLCγ = phospholipase Cγ
RNA = ribonucleic acid
SSRI = selective serotonin reuptake inhibitor
SWA = slow-wave activity
TrkB = tropomyosin-related kinase B

List of original publications

This thesis is based on the following publications:

I **Rosenholm M**, Paro E, Antila H, Võikar V, Rantamäki T: Repeated brief isoflurane anesthesia during early postnatal development produces negligible changes on adult behavior in male mice. PLoS ONE 12, 2017

II Theilmann W*, **Rosenholm M***, Hampel P, Löscher W, Rantamäki T: Lack of antidepressant effects of burst-suppressing isoflurane anesthesia in adult male Wistar outbred rats subjected to chronic mild stress. PLoS ONE 15, 2020 (*=*equal contribution*)

III Kohtala S*, Theilmann W*, **Rosenholm M**, Penna L, Karabulut G, Uusitalo S, Järventausta K, Yli-Hankala A, Yalcin I, Matsui N, Wigren HK, Rantamäki T: Cortical excitability and activation of TrkB signaling during rebound slow oscillations are critical for rapid antidepressant responses. Mol Neurobiol 56, 2019 (*=*equal contribution*)

IV Kohtala S, Theilmann W, **Rosenholm M**, Müller HK, Kiuru P, Wegener G, Yli-Kauhaluoma J, Rantamäki T: Ketamine-induced regulation of TrkB-GSK3β signaling is accompanied by slow EEG oscillations and sedation but is independent of cis-hydroxynorketamine metabolites. Neuropharmacol 157, 2019

Author's contribution to the publications included in this thesis:

I MR participated in treatments and behavioral experiments, data analysis and interpretation, figure preparation, and manuscript writing and editing.

II MR performed the BDNF ELISA analysis of the samples and participated in data analysis and interpretation, figure preparation, and manuscript writing and editing.

III MR participated in pharmacological treatments, sample collection, Western blot analysis of the samples, and EEG surgeries and measurements. This publication is included also in the doctoral dissertation of Samuel Kohtala (University of Helsinki).

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1. Introduction

Major depressive disorder (MDD) is among the most prevalent mood disorders, affecting almost 350 million individuals worldwide (Smith, 2014). The high prevalence and debilitating symptoms make MDD one of the largest contributors to the global disease burden (Olesen et al., 2012; Wang et al., 2003). Depression is also one of the most common causes of early retirement and suicide. The complexity and heterogeneity of MDD poses a particularly difficult challenge for the study of the disease pathophysiology.

Standard treatments for MDD include psychotherapy and monoaminergic antidepressants, such as tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs). However, the clinical efficacy of these drugs is limited, and their antidepressant effects commonly emerge only after weeks of treatment (Insel & Wang, 2009; Rush et al., 2006). This poses a particularly serious problem if the patient is at an acute risk of suicide. Furthermore, approximately one third of patients do not achieve clinical remission with prescription antidepressants. Despite decades of drug development, electroconvulsive therapy (ECT) has remained the most potent antidepressant treatment option since its introduction in the treatment of psychiatric disorders in the 1930s (Lisanby, 2007). The use of ECT, however, is associated with cognitive side effects, and has only limited availability due to the need for an inpatient treatment setting. ECT treatment typically also requires several weekly administrations to achieve and maintain its therapeutic effect, although more rapid antidepressant effects have been observed (Fligelman et al., 2016; Petrides et al., 2011). Sleep deprivation is another somatic treatment known to rapidly alleviate depressive symptoms. The antidepressant effects of sleep deprivation are considerably transient, and the symptoms commonly re-emerge after a subsequent sleep period (Giedke & Schwärzler, 2002; Wu & Bunney, 1990). There is, therefore, a substantial unmet medical need for novel, more effective, and rapidly acting antidepressants with more sustainable therapeutic effects. Interestingly, increasing evidence suggests that diverse anesthetic agents hold such potential.

Anesthetics produce dose-dependent insensateness and loss of consciousness, and they have been widely used in different medical operations (e.g., surgery) since the 19th century (Campagna et al., 2003). While anesthetics are generally considered safe and well tolerated, anesthesia, particularly during early development, has been associated with neuroapoptosis and learning disabilities later in life, raising concerns about its safety for juveniles (Wilder et al., 2009). In adult patients, however, emerging clinical evidence suggests that brief anesthesia may rapidly alleviate depressive symptoms (Tadler & Mickey, 2018). Putative rapid antidepressant effects of anesthesia have been investigated since the 1980s, encouraged by the association of the postictal (i.e., after seizure) slowing of electroencephalogram (EEG) activity (a typical electrophysiological signature during anesthesia), and the antidepressant effects of ECT (Langer et al., 1985; Sackeim et al., 1996). Deep burst-suppressing isoflurane anesthesia

demonstrated rapid antidepressant effects in the early small-scale clinical trials, but the subsequent findings remained inconsistent (Carl et al., 1988; Greenberg et al., 1987; Langer et al., 1985, 1995). However, recent clinical and preclinical findings have renewed the interest in the antidepressant effects of deep anesthesia (Antila et al., 2017; Brown et al., 2018a; Mickey et al., 2018; Weeks et al., 2013).

A major breakthrough in antidepressant treatment emerged at the beginning of the millennia when the rapid antidepressant effects of ketamine were discovered (Berman et al., 2000). Ketamine is a dissociative anesthetic targeting the N-methyl-D-aspartate receptors (NMDAR), but its antidepressant effects become evident particularly at low subanesthetic doses (Berman et al., 2000; Zarate et al., 2006a). Remarkably, the positive effects of ketamine on the core symptoms of depression emerge within hours after administration, with peak effects commonly observed a day after drug delivery. In 2019, an intranasal formulation of (S)-enantiomer of ketamine (esketamine) received approvals from the U.S. Food and Drug Administration and the European Medicines Agency for use as an adjunct to oral antidepressants in the treatment of depression. However, the antidepressant effects of ketamine are usually short-lived, lasting typically only up to a week. The clinical use of ketamine in psychiatry is further limited by its acute psychotomimetic effects and misuse potential.

Several hypotheses have been proposed to underlie the antidepressant effects of ketamine, but the precise cellular and neurobiological mechanisms remain debated. A deeper understanding of the neurobiological basis of rapid antidepressant action would undoubtedly provide new tools for the development of more rapid-acting, safe, and effective antidepressant treatment options. In this literature review, I summarize the current knowledge regarding mechanisms of diverse rapid-acting antidepressants and discuss the effects of anesthesia on neuronal function in the adult and developing brain.

2. Review of the literature

2.1. Major depressive disorder — need for rapid-acting antidepressants

Major depressive disorder is a highly prevalent and debilitating mood disorder. Nearly one in six individuals suffers from MDD during their lifetime in the US (Kessler et al., 2003). Typical symptoms of MDD include persistently depressed mood, anhedonia, low self-esteem, feelings of worthlessness, and impaired sleep, appetite, and sexual function (Nestler et al., 2002). MDD has a high comorbidity with many other psychiatric disorders, including anxiety and substance abuse (Kessler et al., 2003), and also with various other diseases, such as cardiovascular disease and type 2 diabetes (Knol et al., 2006; Musselman et al., 1998). MDD is a multifactorial and heterogenous disorder, where patients express varying symptoms and responses to available treatments (Nestler et al., 2002). Therefore, instead of being a single disease, MDD has been proposed to consist of many syndromes with differing characteristic sets of causes and symptoms (Nestler et al., 2002).

There are currently no sensitive and objective biomarkers of MDD. Instead, the disorder is diagnosed based on clinical assessment and symptomatology. Essentially, the diagnostic criteria, specified in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders requires the presence of five or more specified symptoms for at least two weeks (Otte et al., 2016). Common rating scales for depression severity include questionnaires through which the clinician rates the severity of the patient's depression: a 17-item Hamilton rating scale for depression (Hamilton, 1960) and a 10-item Montgomery-Åsberg Depression Rating Scale (Montgomery & Åsberg, 1979).

Our understanding of MDD pathophysiology remains limited, but both genetic and environmental risk factors seem to be involved. Genetic predisposition plays a role in susceptibility for MDD, although no particular genes have been consistently identified (López-León et al., 2008; Sullivan et al., 2000). MDD is also nearly twice as common in women than in men, but the neurobiological factors contributing to this risk remain unknown (Kessler et al., 1993). Stressful life events and emotional trauma pose a major risk factor for developing MDD (Kendler et al., 1999; Nestler et al., 2002; Vythilingam et al., 2002). First depressive episodes most commonly emerge between mid-adolescence and the mid-40s, with the majority of patients experiencing their first episode before their mid-20s (Malhi & Mann, 2018).

Imaging studies have identified specific neuropathological changes and neuronal circuits to be associated with MDD (Drevets, 2000; Sheline, 2003). Structural abnormalities have been observed, particularly in the highly interconnected limbic regions of the brain that regulate emotional processing and reward. Alterations in neuronal and glial morphology and decreased volume in specific prefrontal and hippocampal regions in depressed patients are commonly

reported (Bremner et al., 2000; Coffey et al., 1993; Drevets et al., 1997; Frodl et al., 2002; Mervaala et al., 2000; Rajkowska et al., 1999; Schmaal et al., 2017; Sheline et al., 2003). In addition, alterations in neuronal network connectivity, dysfunction of the hypothalamic-pituitary-adrenal axis, and increased cytokine levels have been consistently observed (Rosenblat et al., 2014).

Despite our limited knowledge of depression pathophysiology, several forms of treatments are available. Cognitive and behavioral psychotherapy may be sufficient in the treatment of mild-to-moderate depression, but various antidepressant drugs are also widely used (Nestler et al., 2002). Discovery of the antidepressant effects of imipramine and iproniazid, initially studied in the 1950s in the treatment of allergic symptoms and tuberculosis, respectively, paved the way for the current prescription antidepressants (Castrén, 2005). Both imipramine and iproniazid were shown to increase monoaminergic neurotransmission, especially serotonergic and noradrenergic, by either blocking the reuptake of serotonin and noradrenaline to presynaptic nerve endings or by inhibiting the function of monoamine oxidase, an enzyme responsible for monoamine breakdown. Antidepressants that were developed following these findings include tricyclic antidepressants (e.g., amitriptyline), monoamine oxidase A inhibitors (MAOIs, e.g., moclobemide), SSRIs (e.g., fluoxetine, citalopram), selective serotonin and noradrenaline reuptake inhibitors (e.g., venlafaxine), and agomelatine. Similarly to imipramine and iproniazid, these drugs mainly act by increasing the availability of serotonin and/or noradrenaline in the synaptic cleft. Agomelatine is a notable exception in that it functions as an antagonist on serotonin receptor subtypes 5-HT_{2B} and 5-HT_{2C} and as an agonist on melatonin receptors MT_1 and MT_2 (San & Arranz, 2008). More recently developed antidepressant drugs have fewer side effects than the initial tricyclic antidepressants, but the overall efficacy of antidepressants has not significantly improved upon introduction of these new compounds (Nestler et al., 2002). Importantly, about one third of patients remain resistant to treatment with classical antidepressants, emphasizing an unmet medical need for better treatment options (Insel & Wang, 2009).

The clinical antidepressant effects of drugs that enhance monoaminergic neurotransmission led to the formulation of a monoamine hypothesis of depression that postulates disturbances in monoaminergic neurotransmission to underlie the condition (Schildkraut, 1965). However, accumulating clinical and preclinical findings have made it obvious that mere monoamine deficiency is insufficient to explain the pathophysiology of depression and antidepressant action (Krishnan & Nestler, 2008; Manji et al., 2001). Indeed, reduced monoamine function does not consistently produce depressive symptoms in healthy people (Booij et al., 2003). Moreover, monoaminergic antidepressants cause an acute effect in monoaminergic neurotransmission, while the impact on the core symptoms of depression emerges only gradually after chronic treatment (Nestler et al., 2002). Therefore, much of the later research efforts have focused on the long-term neurobiological adaptations induced by antidepressants. Here, the effects of antidepressants on neurotrophic signaling, particularly those regulated by

the brain-derived neurotrophic factor (BDNF), have provided a more thorough understanding of the long-term effects of antidepressants.

2.2. BDNF and antidepressant action

2.2.1. Basic neurobiology of BDNF

BDNF belongs to the neurotrophin family of neurotrophic factors (Autry & Monteggia, 2012; Park & Poo, 2013; Thoenen, 1995). Other neurotrophic factors in this family are nerve growth factor and neurotrophins 3 and 4. BDNF is the most prevalent neurotrophin in the mammalian brain, and its expression is tightly regulated by neuronal activity (Thoenen, 1995). It is an important promoter of neuronal survival, growth, differentiation, and maturation in the developing brain, and a key regulator of neuroplasticity in the adult brain (Park & Poo, 2013). Neuroplasticity refers to the adaptive ability of the brain to change in response to extrinsic and intrinsic stimuli. In the adult brain, this is mainly achieved by activity-dependent modulation (i.e., strengthening or weakening) of synaptic connections or the formation of new connections through synaptogenesis. These mechanisms are involved in the formation of functional synapses during early development, but they are also proposed to be involved in learning and memory processes in adulthood (Lu et al., 2008). Neuroplastic changes may also include neurogenesis, that is, the formation of new neurons. However, in the adult brain, neurogenesis is limited to markedly few brain areas: the subventricular zone of lateral ventricles and the subgranular zone of the dentate gyrus (Zhao et al., 2008).

In the developing brain, BDNF promotes neuronal survival by initiating intracellular signaling pathways that protect neurons from apoptosis (Reichardt, 2006). During axon-dendrite differentiation of developing neurons, BDNF has been shown to promote neurite differentiation into an axon (Shelly et al., 2007). Furthermore, BDNF secretion from target tissue guides neurite outgrowth (Autry & Monteggia, 2012). Involvement of BDNF in adult neurogenesis has been demonstrated in rats by hippocampal infusion of BDNF, which increased the number of adult-born neurons in the subgranular zone (Scharfman et al., 2005). BDNF has been shown to stimulate the formation of functional excitatory and inhibitory synapses *in vitro* (Vicario-Abejón et al., 1998). It has also been strongly implicated in long-term potentiation (LTP), a form of activity-dependent strengthening of synaptic function considered to underlie learning and memory (Lu et al., 2008). Various studies have demonstrated that LTP induced by high-frequency stimulation of neurons is strengthened in the presence of exogenous BDNF and weakened when normal BDNF function is disturbed (Figurov et al., 1996; Korte et al., 1995; Minichiello et al., 2002).

The crucial role of BDNF in neuronal development is further emphasized by observations made in knockout rodents. Homozygous deletion of the BDNF encoding gene in mice leads to severe abnormalities in brain development (Ernfors et al., 1994). These mice express

widespread neuronal atrophy and deficits in peripheral neuronal innervation. The full knockout rodents die soon after birth, mainly before the second postnatal week, due to cardiac and respiratory problems (Erickson et al., 1996; Ernfors et al., 1994). However, further insight into the role of BDNF in the adult brain has been obtained from conditional and inducible BDNF knockout mouse models. These models allow deletion of BDNF in a regionally and temporally specific manner, thereby circumventing lethal developmental abnormalities caused by conventional BDNF knockout (Monteggia et al., 2004; Rios et al., 2001). Conditional BDNF knockout in post-mitotic neurons of mice leads to a substantial postnatal reduction of BDNF in cortical, hypothalamic, and hippocampal regions (Rios et al., 2001). The conditional knockout animals express an increase in anxious behavior, food intake, and body weight. In contrast, mice with inducible BDNF deletion in post-mitotic neurons of broad forebrain regions demonstrate impairments in hippocampal-dependent learning and memory, both when the deletion is timed in early development and in adulthood (Monteggia et al., 2004). The same study also found the inducible BDNF knockout during early life to result in hyperactive behavior of the adult mice.

Additional information on the role of BDNF is provided by naturally occurring single nucleotide polymorphisms in the *Bdnf* gene. The Val66Met polymorphism that substitutes valine to methionine at codon 66 ($BDNF^{Val66Met}$) is of particular interest as it has been shown to impair intracellular trafficking and activity-dependent secretion of BDNF without altering the total levels of BDNF in the brain (Chen et al., 2004, 2006; Egan et al., 2003). The Met allele, which nearly a third of the world's population is estimated to carry (Chen et al., 2006), has been associated with modest memory impairments and decreased hippocampal volume in humans (Egan et al., 2003; Hajek et al., 2012). In rodents, $BDNF^{Val66Met}$ polymorphism has been associated with decreases in hippocampal volume and increased anxiety-like behavior in open field and elevated plus-maze (EPM) tests, with Met/Met homozygotes demonstrating more significant perturbations than Val/Met heterozygotes (Chen et al., 2006).

BDNF is initially synthesized as a precursor form, pro-BDNF, which may then undergo proteolytic cleavage to mature BDNF (Park & Poo, 2013). BDNF exerts its diverse effects through binding to its high-affinity tropomyosin-related kinase B (TrkB) receptor tyrosine kinase (Huang & Reichardt, 2001; Takei et al., 2001). Binding of a BDNF dimer to membrane-bound TrkB receptors induces receptor dimerization that leads to autophosphorylation of several tyrosine (Y) residues in the intracellular tyrosine kinase domain of the receptors, including Y515 and Y816 residues (Minichiello, 2009). Phosphorylation of these tyrosine residues further leads to recruitment of adaptor proteins and activation of intracellular signaling cascades involved in cell survival, differentiation, and plasticity. These include the phospholipase Cγ (PLCγ), the mitogen-activated protein kinase (MAPK), and the phosphatidylinositol 3-kinase (PI3K) pathways (**Figure 1**). Activation of the PLCγ pathway by phosphorylation of the Y816 site generates the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) (Finkbeiner et al., 1997). IP_3 releases intracellular Ca^{2+} from the endoplasmic reticulum that can then activate calcium/calmodulin-dependent protein kinase

IV (CaMKIV)-dependent signaling and protein synthesis through the transcription factor cyclic AMP response element binding protein (CREB). CREB can also be phosphorylated by sequential activation of the small G protein Ras and protein kinases MAPK and Rsk, following TrkB activation (Huang & Reichardt, 2001). The PI3K pathway, activated by TrkB phosphorylation in the Y515 site, leads to downstream activation of protein kinase B (Akt) and mammalian target of rapamycin (mTOR), a major modulator of protein translation, which exerts its translational control through phosphorylation of kinases 4EBP1 and p70S6K further downstream (Takei et al., 2004).

BDNF can also bind to the truncated form of TrkB receptor TrkB.T1 (Park & Poo, 2013). TrkB.T1 lacks the intracellular tyrosine kinase domain, and hence works as a dominant negative regulator of TrkB signaling. Moreover, pro-BDNF can elicit TrkB-independent signaling by binding to the p75 neurotrophin receptor (p75NTR) (Autry & Monteggia, 2012). Signaling initiated by p75NTR regulates cellular responses opposite to those regulated by TrkB, including neuronal apoptosis (Bamji et al., 1998; Friedman, 2000). Therefore, the cleavage of secreted neurotrophins is a crucial determinant of the resulting neurotrophic function.

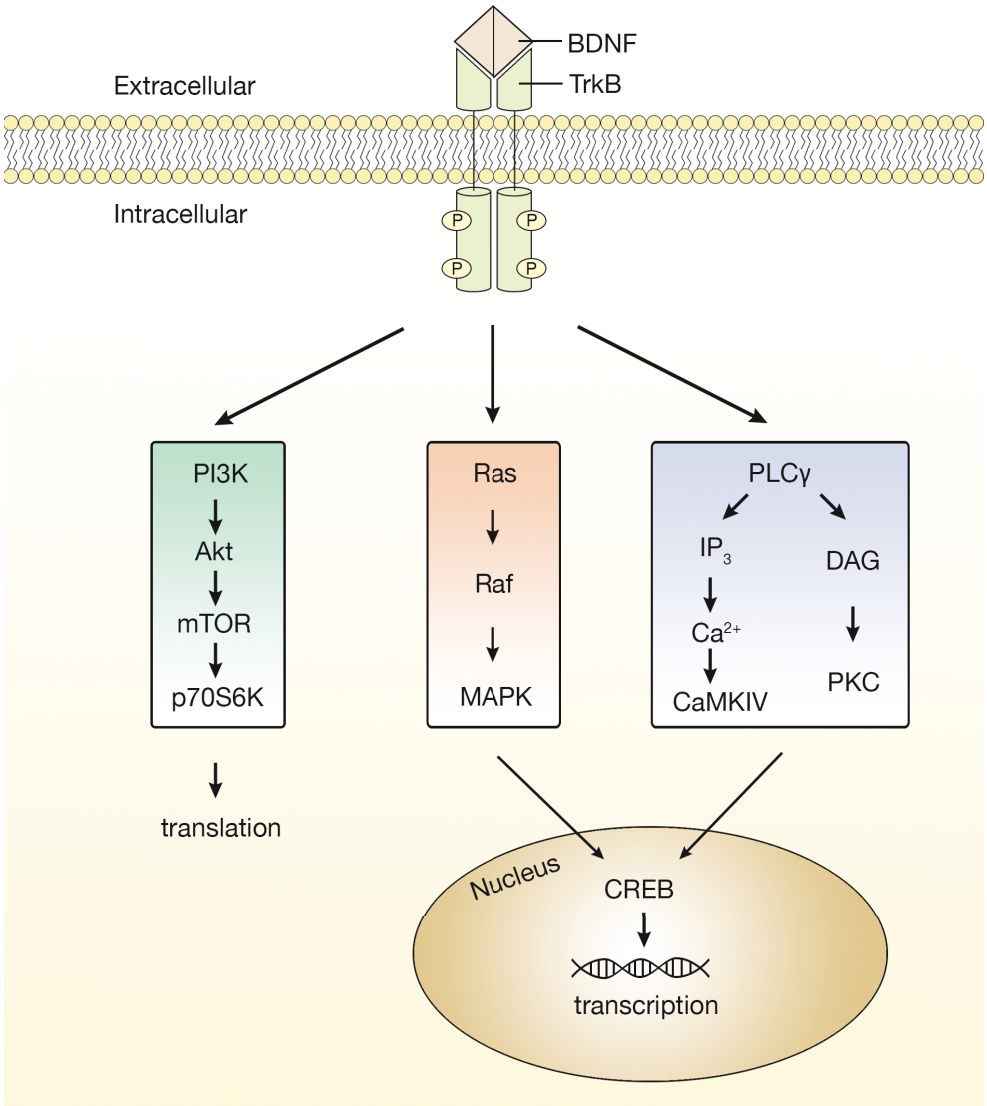

Figure 1. Intracellular signaling mechanisms activated by TrkB neurotrophin receptor. PI3K, MAPK and PLCγ signaling pathways are activated by the dimerization and subsequent autophosphorylation of TrkB. The pathways influence diverse neuronal functions, such as cell survival, and transcription and translation of synaptic proteins.

2.2.2. Neurotrophin hypothesis of depression

Accumulating evidence suggests that alterations in BDNF signaling play a major role in depression pathophysiology and antidepressant function. Various findings have contributed to the formulation of a neurotrophic hypothesis of antidepressant action (Duman et al., 1997; Duman & Monteggia, 2006). The hypothesis postulates that a deficiency in neurotrophic support that results in neuronal atrophy and circuit dysfunction underlies depression pathophysiology, and that antidepressant drugs act by reversing these deficiencies. This hypothesis is supported by a large body of experimental findings reviewed here.

Brain imaging studies have revealed various structural and morphological abnormalities in the brain to be associated with MDD (Price & Drevets, 2010). In particular, decreased gray matter volume in specific cortical and limbic areas has been consistently reported in depressed patients (Drevets et al., 1997; Rajkowska et al., 1999; Sheline, 2003). In rodents, exposure to chronic stress and other depression models has produced atrophic morphological alterations in cortical areas and the hippocampus (HC) reminiscent of those observed in depressed patients (Li et al., 2011; Liu & Aghajanian, 2008; McEwen, 2005; Moda-Sava et al., 2019).

Various stressors, such as repeated immobilization stress, foot shocks, and unpredictable chronic mild stress (CMS) have been shown to decrease *Bdnf* mRNA expression in the rodent HC and prefrontal cortex (PFC) (Nibuya et al., 1999; Rasmusson et al., 2002; Smith et al., 1995). In humans, decreased BDNF levels have been measured in the serum of depressed patients and in the PFC and HC of suicide victims (Dwivedi et al., 2003; Karege et al., 2002). Moreover, the Met allele has been associated with a higher susceptibility for MDD, although contrasting findings have also been reported (Gatt et al., 2009; Jiang et al., 2005).

Monoaminergic antidepressants have been consistently shown to induce synaptic plasticity and neurogenesis in the adult central nervous system (CNS), stimulating research interest in the role of BDNF in antidepressant treatment (Duman et al., 2016; Rantamäki & Yalcin, 2016). Chronic—but not acute— administration of various antidepressants, including fluoxetine and reboxetine, induced neurogenesis in the rat HC (Malberg et al., 2000). A further study reported hippocampal neurogenesis to be necessary for the antidepressant effects of fluoxetine, since a disruption of neurogenesis by hippocampal irradiation abolished the antidepressant-like behavioral effects in the novelty suppressed feeding test in mice (Santarelli et al., 2003). Regulation of BDNF expression has been strongly implicated in the antidepressant effects of classical antidepressants. Chronic—but not acute— administration of diverse monoaminergic antidepressants, including imipramine and sertraline, for 21 days increased the expression of *Bdnf* and *TrkB* mRNA in the rat HC (Nibuya et al., 1995). Increased hippocampal *Bdnf* expression was also reported after only two days of tranylcypromine (MAOI) treatment (Russo-Neustadt et al., 2000). The effects were even more pronounced after a one-week chronic administration. Diverse antidepressants, including imipramine, fluoxetine, and citalopram, were also shown to acutely increase TrkB phosphorylation in the rodent HC and

cortical regions (Rantamäki et al., 2007; Saarelainen et al., 2003). Furthermore, post-mortem brain tissue collected from depressed subjects treated with antidepressants showed increased BDNF expression in various hippocampal regions (Chen et al., 2001).

More direct evidence for the importance of BDNF in antidepressant effects is provided by studies demonstrating that intrahippocampal and intracerebroventricular infusions of BDNF produce antidepressant-like behavioral effects in the forced swim test (FST) and the learned helplessness model in rodents (Hoshaw et al., 2005; Shirayama et al., 2002; Siuciak et al., 1996). Several reports have also indicated normal BDNF and TrkB function to be necessary for an antidepressant-like behavioral response to monoaminergic antidepressants in rodents. Disruption of regular TrkB signaling by an overexpression of the dominant negative TrkB.T1 isoform in postnatal cortical and hippocampal neurons abolished antidepressant-like behavioral effects of fluoxetine and citalopram in the mouse FST (Rantamäki et al., 2007; Saarelainen et al., 2003). In addition, viral vector-mediated localized BDNF knockout in the mouse HC resulted in a loss of antidepressant-like behavioral effects of classical antidepressant drugs desipramine and citalopram in FST (Adachi et al., 2008). Furthermore, conditional deletion of the TrkB encoding gene in neural progenitor cells but not in differentiated cells in the mouse dentate gyrus abolishes the antidepressant-like behavioral responses of chronic fluoxetine and imipramine administrations, indicating a crucial role for TrkB-mediated hippocampal neurogenesis in antidepressant response (Li et al., 2008).

Overall, these findings support the idea postulated in the neurotrophic hypothesis of depression proposing that diminished neurotrophic support underlies depressive symptoms, whereas antidepressants function by renormalizing this imbalance. However, depression etiology likely exceeds mere chemical imbalance in the brain, be it monoamines or neurotrophins, and the focus of antidepressant research has lately expanded its scope to understand the potential functional benefit of antidepressants' ability to regulate neuroplasticity.

2.2.3. Network hypothesis of depression

Despite BDNF being acknowledged as a crucial mediator of neuroplasticity in the brain, the diverse manners through which the brain changes can hardly be considered to stem from the expression of a single molecular entity. Instead, synaptic and neuronal plasticity involves activity-dependent morphological and functional changes in neuronal networks that are influenced by environmental input (Hensch, 2005). These aspects are taken into consideration in a proposed network hypothesis of depression that suggests disturbances in the information processing of neuronal circuits underlie depression (Castrén, 2005; Castrén & Rantamäki, 2010). This hypothesis postulates that antidepressants elicit their therapeutic effects by enabling a gradual functional reorganization of pathologically affected circuits through activity-dependent modifications in neuronal networks.

The mammalian primary visual cortex has been extensively utilized to provide insight into activity-dependent functional neuroplasticity and the network hypothesis of depression. The visual system is particularly vulnerable to experimental manipulations as animals are relatively easy to partially or completely deprive of visual stimulus by suturing an eyelid shut or by manipulating surrounding illumination (e.g., by dark rearing). Indeed, monocular deprivation during a specific temporal window during early postnatal development can cause a severe and persistent deficit in vision (i.e., amblyopia) in the deprived eye due to improper visual input (Hensch, 2005; Wiesel & Hubel, 1963). The lack of stimulus from the deprived eye results in a shift in ocular dominance distribution of the visual cortex in favor of the open eye, thereby functionally disconnecting the deprived eye from the visual cortex. Monocular deprivation does not have similar effects when applied outside a specific developmental window of heightened plasticity (i.e., sensitive period).

The amblyopia model has provided remarkable insight into antidepressant function, supporting the network hypothesis. Chronic fluoxetine administration in combination with patching of the non-amblyopic eye (cf. rehabilitation), but neither alone, restored vision both behaviorally (visual acuity) and functionally (visually evoked potentials in the visual cortex) in an amblyopic eye in adult rats (Maya-Vetencourt et al., 2008). These effects were accompanied by increased BDNF expression in the visual cortex. The network hypothesis has also been tested using the fear conditioning paradigm (Karpova et al., 2011). Fear extinction training during early development has been shown to bring permanent erasure of fear memory, while, in adult animals, the effects are more transient and vulnerable to renewal (Kim & Richardson, 2010). However, chronic fluoxetine administration in combination with extinction training resulted in a more effective erasure of fear memory and absence of fear renewal than either of the treatments alone (Karpova et al., 2011). The same study found the effects to be absent in heterozygous $BDNF^{+/-}$ mice. These findings suggest that fluoxetine can reinstate a juvenile-like form of neuronal plasticity, even in the adult brain.

In light of our understanding of depression as a gradually developing disorder, it is logical to assume that antidepressant treatments also require weeks for the therapeutic effects to manifest (O'Leary et al., 2014). The network hypothesis provides a plausible basis for antidepressants' delayed onset of action. This is because BDNF-induced changes in synaptic plasticity and activity-dependent rewiring of neuronal connections require slowly developing adaptations before the effects on complex behaviors and mood become evident. Notably, the network hypothesis also helps to explain why psychotherapy and antidepressant medication provide a more effective therapeutic response in combination as the hypothesis emphasizes the importance of external stimulus in addition to the acute drug effects (Pampallona et al., 2004). However, these views are challenged by the surprisingly rapid alleviation of depressive symptoms that can be achieved with some treatments. These methods include somatic treatments, such as ECT and sleep deprivation, but various pharmacological options have also emerged. Interestingly many of these are anesthetics.

2.3. Diverse developmental stage-dependent effects of anesthesia

The discovery of anesthesia revolutionized clinical medicine, allowing complex surgical procedures while the patient remains disconnected from painful stimulus (Franks, 2008). Anesthesia, by definition, produces reversible, dose-dependent loss of consciousness, analgesia, and insensateness. To achieve balanced surgical anesthesia, a multimodal approach is used in which anesthetic compounds are co-applied with antinociceptive agents, such as opioids, muscle relaxants, cardiovascular drugs, and ventilatory and thermoregulatory support (Brown et al., 2018b). This approach allows for sufficient depth of anesthesia while minimizing the required amount of a single drug, thereby decreasing the risk for drug-related side effects. Today, anesthesia is administered to tens of millions of people every year. However, as the use of anesthesia increases, so does our understanding of the diverse influences of anesthetics on brain function that exceed its conventional pharmacological effects.

Anesthetics are a pharmacologically diverse class of compounds that primarily act by facilitating neuronal inhibition through $GABA_A$ receptor ($GABA_AR$) function (e.g., isoflurane, sevoflurane) and/or suppressing neuronal excitation by inhibiting the glutamatergic NMDAR activity (e.g., ketamine, nitrous oxide) (Campagna et al., 2003). $GABA_A$ receptors are chloride ion permeable channels activated by gamma-aminobutyric acid (GABA), and are one of the main receptors responsible for inhibitory synaptic transmission in the adult brain. In addition to $GABA_A$Rs and NMDARs, some anesthetics also inhibit glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), nicotinic acetylcholine receptors, and various ion channels. Moreover, α_2-adrenergic autoreceptor agonists, such as (dex)medetomidine, have been introduced as an adjunct for general anesthesia due to their capability to produce anxiolysis, analgesia, and sedation (Correa-Sales et al., 1992). These compounds act by hyperpolarizing and decreasing the release of noradrenaline from locus coeruleus neurons, thereby inducing sleep-like sedation (Brown et al., 2011).

In the spinal cord, an anesthetic-induced overall decrease in neuronal excitability reduces the transmission of noxious stimuli (Campagna et al., 2003). In the brain, anesthetics globally decrease cerebral blood flow and glucose metabolism, accompanied by a generalized slowing of EEG activity, that is, a power increase in low-frequency, high-amplitude delta oscillations (1–4 Hz). Anesthesia therefore shares many behavioral, functional, and EEG features with deep sleep, and the same subcortical networks that regulate sleep may be involved in anesthetic action as well (Brown et al., 2010). While it is notable that at low doses (e.g., during induction) anesthetics may induce paradoxical excitation, as the level of anesthesia deepens, low-frequency, high-amplitude EEG oscillations increase. Increasing the depth of anesthesia eventually leads to a burst-suppressing EEG state, which is not observed under physiological sleep. Burst-suppression is a state characterized by alternating patterns of quiescent EEG activity followed by brief high-frequency bursting activity.

Most general anesthetics, such as volatile halogenated hydrocarbons (e.g., isoflurane, sevoflurane), have relatively short half-lives, which allows for rapid recovery following drug discontinuation (Campagna et al., 2003). General anesthetics are subject to hepatic metabolism, a notable exception being nitrous oxide (N_2O), which is eliminated without significant metabolism through respiration (Nagele et al., 2018). Common side effects of general anesthetics include cardiopulmonary depression and emergence delirium upon anesthesia cessation. In addition, NMDAR antagonists, such as ketamine and N_2O, are characterized by psychotomimetic effects that precede the anesthetic state (Franks, 2008). Moreover, brain activity during NMDAR antagonist anesthesia differs drastically from that observed with $GABA_A$ targeting anesthetics, as evidenced by their opposite effects on global cerebral blood flow and thalamic glucose metabolism (Långsjö et al., 2005).

While general anesthetics were initially considered to be safe and well-tolerated compounds with reversible effects, accumulating evidence has shown that exposure to anesthetics, particularly during early stages of brain development, can cause long-lasting disturbances in neurocognitive function later in life (Vutskits & Xie, 2016). This is particularly alarming because millions of newborns are exposed to anesthetics every year. However, increasing evidence shows that the depth, duration, and time points of anesthesia exposure have a major influence on the potentially detrimental effects of anesthetics as several studies have demonstrated that in adults, anesthetics may even hold therapeutic potential against some disorders, including MDD (Vutskits, 2018).

2.3.1. Anesthetics and the developing brain

Early brain development is a carefully orchestrated and multi-staged process that progresses from the generation and proliferation of progenitor cells through the migration and differentiation of neurons and glia to the subsequent branching of axons and dendrites, and the eventual synaptogenesis and formation of neuronal networks that give rise to complex behaviors (**Figure 2**) (Andersen, 2003). The basis for the neuronal network structure is laid down during embryonic and fetal development through intrinsic developmental mechanisms, but the structural development of neuronal circuitry continues after birth and through adolescence in an experience- and activity-dependent manner (Hensch, 2004, 2005; Rice & Barone, 2000).

Brain development generally follows similar sequences across different mammalian species, but the timing and speed of the process can vary dramatically between species (Clancy et al., 2001). For instance, the formation of the neural tube, one of the first steps of CNS development *in utero*, occurs approximately halfway through a 3-week gestation in rodents, whereas in humans it takes place between embryonic weeks 3 and 4, approximately one month into a 9-month gestation (DeSesso et al., 1999). Neural tube formation is followed by a sequence of developmental processes, such as cell proliferation, migration, differentiation, synaptogenesis,

and myelination, that take place over a timeline of days in rodents versus months in humans (**Figure 2-3**) (Rice & Barone, 2000). These interspecies differences provide a particular challenge in translating preclinical animal findings to humans.

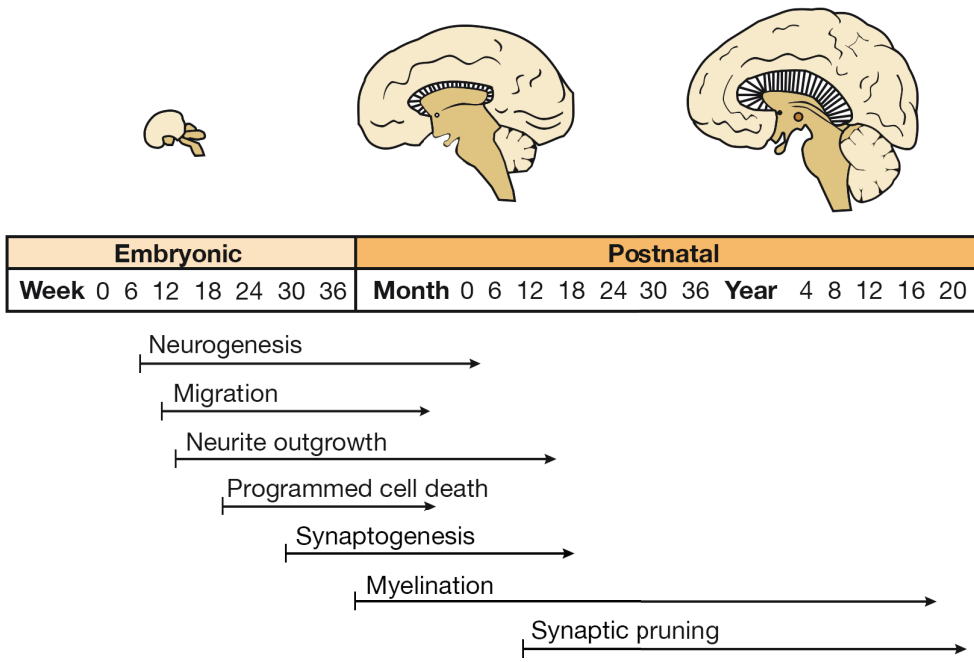

Figure 2. Phases of early brain development in humans. The structural basis of the brain is already formed during embryonic development, but the activity-dependent fine-tuning of neuronal networks continues throughout postnatal life and adolescence. Figure modified after Andersen (2003).

During neurogenesis in embryonic development, neurons are initially overproduced and are eventually eliminated to about 50% of their initial amount through programmed cell death (Andersen, 2003). A similar event also occurs during postnatal synaptogenesis, when a significant number of synapses are formed, most of which are pruned out before adolescence (Huttenlocher, 1979). These morphological overshoots likely serve to enable activity-dependent and selective elimination of redundant synaptic connections and strengthening of the remaining connections, thereby increasing the efficiency and selectivity of synaptic transmission (Andersen, 2003; Kano & Hashimoto, 2009).

The developing brain is particularly susceptible to various disturbances, and many psychiatric disorders are believed to have a developmental origin (Andersen, 2003). Such disturbances include environmental, genetic, and pharmacological factors. These extrinsic factors are

especially influential during specific time windows of early development called sensitive periods (Hensch, 2004, 2005). Adversity-driven structural and functional alterations during these periods may have a permanent effect on neuronal circuit function. This phenomenon is particularly well demonstrated in the previously discussed visual cortex model, where a closure of one eye during a sensitive postnatal period leads to a permanent loss of vision in that eye (Section 2.2.3.). However, experiences during critical periods can have a significant impact on the acquisition and learning of a particular skill or behavior (Hensch, 2005). Since different brain areas mature at different stages of prenatal and postnatal development, they also have distinct temporal windows of opportunity and vulnerability. The brain retains some plasticity through adulthood but not in as high abundance as during sensitive periods, a notable example being the gradually decreasing ability of the brain to functionally recover from injuries (Feinberg, 1982).

As the developmental processes in the brain are driven by environmental input in an activity-dependent manner, anesthetics that significantly interfere with excitatory and inhibitory neurotransmission can disturb these processes, particularly during the early stages of postnatal brain development. Indeed, repeated exposure to anesthesia before the age of four years has been associated with increased risk for the development of learning disability later in life (Flick et al., 2011; Wilder et al., 2009). In rodents, long-term effects of early life anesthesia have been shown to vary depending on age of exposure (Briner et al., 2011). Exposure to anesthetics such as isoflurane and sevoflurane during the brain growth spurt at the first postnatal week has been consistently shown to produce increased apoptotic neurodegeneration in cortical, hippocampal, and thalamic areas in rodents (Jevtovic-Todorovic et al., 2003; Tagawa et al., 2014). These effects were associated with sustained behavioral abnormalities in rats, as demonstrated in the Morris water maze test, which measures spatial navigation and memory in rodents (Shen et al., 2013). Ketamine (20–40 mg/kg, s.c.) induced neuroapoptosis in the caudate nucleus and cortex of mice dose-dependently only five hours after administration at postnatal day (P)7 (Young et al., 2005). Moreover, combinations of anesthetics have been shown to produce more deleterious effects than a single agent (Tagawa et al., 2014). Ketamine co-administered with propofol or thiopental induced neuroapoptosis 24 hours after administration in P10 mouse olfactory bulb and stria terminalis (Fredriksson et al., 2007). The treatments also elicited persistent disturbances in motor behavior, spatial learning, and memory when tested later at P55. Sevoflurane administered to rat pups at P7 caused a hippocampal synaptic loss that was more pronounced after repeated anesthesias (3 x 2 hours) than a single 2-hour anesthesia (Amrock et al., 2015).

The rodent brain goes through rapid maturation during the first postnatal weeks. This is reflected in the contrasting effects of anesthesia at different developmental stages. In contrast to the neuroapoptotic effects of anesthesia during the first postnatal week, exposure of rats to anesthetics at P16 increased dendritic spine density instead (Briner et al., 2010; De Roo et al., 2009). Furthermore, propofol decreased dendritic spine density when administered at P5 and P10, but increased it at P15, P20, and P30 (Briner et al., 2011). The effects were sustained until

the rats were up to 3 months old. Similar developmental stage-dependent effects were observed after brief sevoflurane anesthesia (Qiu et al., 2016). A 30-minute sevoflurane anesthesia (2.5%) induced a decrease in dendritic spine density in rat cortical pyramidal neurons when administered at P7 but an increase at P15. Neither of these alterations persisted into adulthood. Time-dependent alterations in isoflurane effect were also observed in organotypic hippocampal slices collected at different postnatal time points (Wise-Faberowski et al., 2005). Isoflurane (1.5%) exposure for 5 hours caused neurodegeneration in slices collected at P7 but not at P4 or P14. Developmental differences in anesthetic response have also been demonstrated in non-human primates. A 24-hour infusion of ketamine was reported to cause neuroapoptosis in the rhesus macaque frontal cortex at P5 but not at P35 (Slikker et al., 2007). Moreover, the same study reported that a 3-hour infusion of ketamine did not result in increased neuroapoptosis, even at P5, indicating the important role of the exposure duration in the apoptotic neurodegeneration.

Overall, several lines of study have demonstrated that during early postnatal life, the developing brain is particularly susceptible to anesthesia-induced morphological and functional alterations that may cause permanent behavioral impairments later in life. However, conflicting evidence exists for the long-term effects of early postnatal anesthesia, and the roles of the depth, time point of exposure, and total exposure duration in the potential permanent effects of anesthesia remain obscure. Moreover, in human studies, the conclusions are limited by confounding factors. As anesthesia during adolescence is rarely delivered without accompanying surgery, the long-term effects on learning and memory may be influenced by the surgery itself or the condition that is being surgically treated. Nevertheless, these detrimental effects seem to be specific to certain sensitive temporal windows during early development, and therefore highly dependent on the time point of exposure to anesthesia (**Figure 3**).

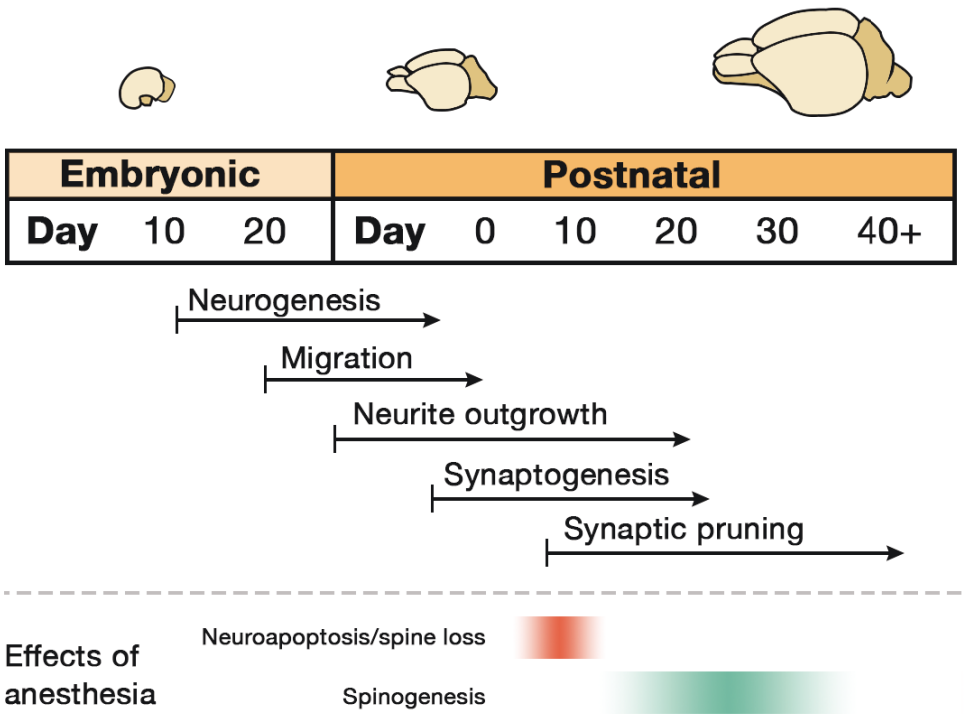

Figure 3. Phases of early brain maturation and the developmental stage-dependent impact of anesthesia in rodents (Farhy-Tselnicker & Allen, 2018; Thion & Garel, 2017)**.** The impact of anesthesia on the developing brain is dependent on the maturation stage during anesthesia exposure. Anesthesia during the first postnatal week in rodents is associated with increased neuroapoptosis, whereas anesthesia during the second and third postnatal weeks lacks such effects and induces synaptogenesis instead.

2.3.2. Antidepressant effects of anesthesia

Interest in the potential antidepressant effects of general anesthetics emerged during the 1980s after the discovery that the postictal electrocerebral silence after ECT may predict the onset of action of ECT's therapeutic effects (Langer et al., 1985). General anesthetics cause a similar silencing of EEG power during anesthesia (Campagna et al., 2003; Clark & Rosner, 1973). Indeed, early preliminary clinical studies investigating the antidepressant potential of isoflurane demonstrated an antidepressant effect of brief, repeated burst-suppressing anesthesia in patients (Carl et al., 1988; Engelhardt et al., 1993; Langer et al., 1985, 1995). In the first small-scale clinical study by Langer and colleagues (1985), isoflurane anesthesia

alleviated depressive symptoms in 9 out of 11 patients. These initial findings were supported by studies comparing the effects of repeated burst-suppressing isoflurane anesthesia to ECT (Carl et al., 1988; Engelhardt et al., 1993). A follow-up study found six consecutive 15-minute burst-suppressing isoflurane anesthesia exposures to elicit a more effective and rapid antidepressant response than ECT, accompanied with fewer cognitive side effects (Langer et al., 1995). However, many subsequent findings with isoflurane and other volatile anesthetics remained inconsistent, and this line of study was inactive for decades (Garcia-Toro et al., 2004; García-Toro et al., 2001; Greenberg et al., 1987).

Recently emerging clinical and preclinical observations have restimulated interest in the antidepressant potential of deep anesthesia (Tadler & Mickey, 2018). Indeed, brief burst-suppressing anesthesia induced by isoflurane or propofol has shown promise in small clinical trials, echoing the early positive findings (Mickey et al., 2018; Weeks et al., 2013). A series of ten 15-minute burst-suppressing isoflurane anesthesia sessions had comparable antidepressant efficacy to ECT in patients with medication-refractory depression. They were also more tolerable than ECT in terms of neurocognitive side effects (Weeks et al., 2013). In another clinical trial, 10 burst-suppressing propofol infusions administered for 15 minutes three times per week rapidly alleviated depressive symptoms (Mickey et al., 2018). These findings are supported by recent preclinical studies demonstrating that a single burst-suppressing isoflurane anesthesia produces antidepressant-like effects in the FST and rodent depression models (Antila et al., 2017; Brown et al., 2018a; Zhang et al., 2019). However, further preclinical and clinical research into the antidepressant effects of deep anesthesia is clearly needed.

2.4. Rapid-acting antidepressant ketamine

In 2000, Berman et al. demonstrated that ketamine, an anesthetic drug and non-competitive antagonist of glutamatergic NMDAR, rapidly alleviated depressive symptoms in a matter of hours, even in treatment-resistant patients, after a single subanesthetic administration (Berman et al., 2000). This groundbreaking finding was replicated in numerous other double-blind, placebo-controlled clinical studies in depressed patients (Murrough et al., 2013; Zarate et al., 2006a). In addition, ketamine rapidly reduced suicidal ideation in depressed patients (Diazgranados et al., 2010; Price et al., 2009; Wilkinson et al., 2018). Ketamine has also shown antidepressant efficacy in patients who do not respond to ECT (Ibrahim et al., 2011). The antidepressant and anti-suicidal effects of ketamine may be sustained for weeks in some individuals but are commonly more transient. The majority of patients who respond to a single infusion of ketamine do not meet response criteria two weeks after administration (Pennybaker et al., 2017).

Ketamine was initially synthesized in 1962 and developed as an anesthetic drug, obtaining approval for clinical use from the FDA in 1970 (Kalmoe et al., 2020). Ketamine was first widely used as a battlefield anesthetic in the Vietnam War before gaining widespread use in

clinical anesthesiology. Ketamine is structurally similar to the hallucinogenic drug phencyclidine (PCP) and belongs to the chemical group of arylcyclohexylamines. It produces dose-dependent anesthesia that differs from the anesthesia induced by non-NMDAR binding agents, such as volatile anesthetics and propofol, in its dissociative quality. The use of ketamine in clinical anesthesia is favored due to its minimal effect on cardiac and respiratory functions.

In off-label use for treating depression, racemic ketamine, consisting of (S)- and (R)-enantiomers, is administered as an intravenous infusion for 40–60 minutes in a subanesthetic dose (0.5 mg/kg) (aan het Rot et al., 2012). This dose is generally well tolerated, but the adverse effects of ketamine and the short duration of its therapeutic effect limit its widespread clinical use (Krystal et al., 2019). Six repeated ketamine infusions have been proven to be safe, well tolerated, and feasible in maintaining the antidepressant effects, but the long-term safety of ketamine requires further investigation (aan het Rot et al., 2010; Murrough et al., 2013). A subanesthetic dose of ketamine may cause dissociative symptoms reminiscent of acute psychosis, which include extreme perturbations in thought and sensory processing, hallucinations, and euphoria. These psychotomimetic effects also present the risk of ketamine's potential misuse since this state is desired in the recreational use of the drug.

Findings of the antidepressant effects of ketamine are supported by a large body of preclinical research demonstrating ketamine's antidepressant-like effects in a variety of animal models of depression, such as learned helplessness and unpredictable CMS (Autry et al., 2011; Li et al., 2011; Li et al., 2010; Lindholm et al., 2012; Maeng et al., 2008; Zanos et al., 2016). Despite extensive preclinical studies, the precise neurobiological mechanisms of ketamine's antidepressant action remain unclear. However, various hypotheses have been proposed, many of which partially align with the neurotrophic and network hypotheses of depression previously discussed.

2.4.1. Neurobiological basis of ketamine's antidepressant action

The neurobiological mechanisms underlying the rapid antidepressant effects of subanesthetic doses of ketamine are complex and have not been fully elucidated. The mechanisms likely exceed mere NMDAR antagonism since other NMDAR antagonists, such as the long-acting antagonist memantine, have proven insufficient in producing rapid antidepressant responses in clinical trials (Newport et al., 2015; Zarate et al., 2006b). Memantine does have a different NMDAR trapping profile than ketamine, but further evidence indicates additional mechanisms to be involved in ketamine's antidepressant action (Kotermanski et al., 2009; Mealing et al., 1999). Indeed, the clinical effects of ketamine are maintained well beyond the acute pharmacological effects of the drug (plasma half-life 2–3 h) and commonly emerge only after the psychotomimetic effects of ketamine have subsided (aan het Rot et al., 2012). This indicates that other mechanisms beside acute NMDAR antagonism are involved in the sustained antidepressant effects.

Despite having an antagonizing effect on glutamatergic NMDAR, subanesthetic doses of ketamine paradoxically increase cortical excitability (Abdallah et al., 2018a; Cornwell et al., 2012; Di Lazzaro et al., 2003). This effect is hypothesized to be caused by ketamine preferentially inhibiting NMDAR in GABAergic inhibitory interneurons, leading to disinhibition and increased firing of glutamatergic pyramidal neurons (**Figure 4A**) (Abdallah et al., 2018b; Homayoun & Moghaddam, 2007). Indeed, subanesthetic doses of ketamine have been indicated to acutely increase glutamatergic neurotransmission in non-NMDA glutamatergic receptors, particularly in AMPA receptors (AMPAR) (Zanos & Gould, 2018). In humans, subanesthetic ketamine infusion caused an increase in prefrontal glutamate release, as measured by magnetic resonance spectroscopy-based detection of ^{13}C-labeled glutamine (Abdallah et al., 2018a). Extracellular levels of glutamate were shown to increase after subanesthetic doses of ketamine (10–30 mg/kg, i.p.) in rat PFC, while the effect was absent at higher doses (50 mg/kg, i.p.) (Moghaddam et al., 1997). In contrast, an anesthetic dose of ketamine (200 mg/kg, i.p.) caused a decrease in extracellular glutamate levels. AMPAR antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) blocked the effects of ketamine in the learned helplessness model and FST in mice (Autry et al., 2011; Maeng et al., 2008) and in learned helplessness, tail suspension test (Koike et al., 2011), and FST in rats (Koike & Chaki, 2014). The disinhibition hypothesis is also supported by a recent electrophysiological study, where a low dose of ketamine reduced inhibitory input to CA1 pyramidal cells in rat hippocampal slices (Widman & McMahon, 2018). Furthermore, subanesthetic ketamine was found to increase calcium transients in cortical pyramidal neurons, as measured by two-photon imaging of head-fixed mice (Ali et al., 2020). In addition to selective action on interneuron NMDARs, ketamine has been proposed to directly block NR2B subunit-containing extrasynaptic NMDARs in pyramidal neurons that are tonically activated by ambient glutamate under basal conditions (**Figure 4B**) (Miller et al., 2016). This blockade is proposed to increase excitatory drive by disinhibiting protein synthesis.

Similarly to its relationship with monoaminergic antidepressants, BDNF has been indicated in the antidepressant-like behavioral effects of ketamine. The antidepressant effects of subanesthetic ketamine (3–10 mg/kg, i.p.) in FST were blocked by inducible knockout of BDNF in mouse forebrain areas (Autry et al., 2011) and in BDNF^{Val66Met} knock-in mice (Liu et al., 2012). A postnatal conditional knockout of TrkB also negated ketamine's behavioral effects (Autry et al., 2011). Furthermore, the same study demonstrated that ketamine and another NMDAR antagonist MK-801 (0.1 mg/kg, i.p.) increased BDNF protein expression in the cortex only 30 minutes after administration, but the effect remained transient and was not observed after 24 hours. An infusion of function-blocking BDNF antibody in the rat medial PFC also blocked the antidepressant-like effects of subanesthetic ketamine (10 mg/kg, i.p.) in FST, suggesting that BDNF release is necessary for ketamine's antidepressant action (Lepack et al., 2014). The same study found that calcium channel antagonists verapamil and nifedipine neutralized ketamine's effects in FST, indicating a role for L-type voltage-dependent calcium channel activation as well. However, another study reported a higher range subanesthetic dose

of ketamine (50 mg/kg, i.p.) to retain its antidepressant-like effects in FST in heterozygous *Bdnf*$^{+/-}$ mice (Lindholm et al., 2012).

Ketamine has also been shown to recruit molecular machinery connected to neurotrophic function. Subanesthetic ketamine (10 mg/kg, i.p.) rapidly induced phosphorylation of mTOR and subsequently increased synaptic protein expression and formation of excitatory synapses in the mouse PFC (Li et al., 2010). Indeed, ketamine also dose-dependently increased phosphorylation of other signaling proteins indicated in BDNF signaling and synapse formation, including 4E-BP1, p70S6K, MAPK, and AKT. However, these signaling effects were transient and had reduced back to baseline levels two hours after ketamine delivery. The signaling effects were dependent on AMPAR and mTOR activation since pretreatment with AMPAR antagonist NBQX or mTOR antagonist rapamycin completely abolished the behavioral and molecular effects of ketamine. After the molecular signaling effects subsided, an increase in the expression of synaptic proteins, including postsynaptic density protein 95 (PSD95), activity-regulated cytoskeleton-associated protein (Arc), synapsin I, and GluR1 was observed. Furthermore, two-photon imaging revealed increased spine density in the apical tuft pyramidal neurons in the medial PFC. Similar mTOR-dependent behavioral and signaling effects were observed with an NMDAR subunit NR2B-specific antagonist, Ro 25-6981. Studies aiming to replicate the findings have, however, been inconsistent, and the role of mTOR pathway in ketamine's antidepressant effect remains debated (Autry et al., 2011; Zanos et al., 2016). Ketamine was also shown to rescue cortical spine loss induced by chronic exposure to corticosterone in the drinking water of mice (Moda-Sava et al., 2019). Interestingly, the effects were selective to spines lost as a result of the corticosterone treatment. However, a majority of spines lost during corticosterone treatment still remained unrestored after ketamine treatment.

The transcriptional and translational regulation of neurons has been consistently shown to be influenced by ketamine. Subanesthetic ketamine (3 mg/kg, i.p.) and MK-801 (0.1 mg/kg, i.p.) rapidly inactivated the phosphorylated form of eukaryotic elongation factor 2 (eEF2), a suppressor of dendritic translation, enabling rapid translation of BDNF transcripts in the mouse HC (Autry et al., 2011). The authors proposed that this inactivation was mediated by ketamine's ability to inhibit spontaneous NMDAR-dependent excitatory postsynaptic currents (**Figure 4C**). Inhibition of the translational control of eEF2 with antagonists rottlerin and NH125 was found to be sufficient to elicit an antidepressant-like behavioral response in FST. Ketamine (15 mg/kg, i.p.) also activated transcription factor CREB in the rat PFC (Réus et al., 2016). Another cell signaling component shown to have significance in ketamine's antidepressant response is glycogen synthase kinase 3 (GSK3), a protein kinase that is expressed in α and β isoforms and whose function is regulated by BDNF through the PI3K signaling pathway (Li & Jope, 2010). GSK3 inhibits the function of transcription factor CREB and is highly active at rest, but phosphorylation of its serine-9 residue inactivates it, disinhibiting CREB. GSK3 has been previously implicated in the mood-stabilizing effect of lithium (Stambolic et al., 1996). A subanesthetic dose of ketamine (10 mg/kg, i.p.) inhibited

GSK3 30 and 60 minutes after administration in mouse PFC and HC (Beurel et al., 2011). In the same study, a knock-in mutation of GSK3 negated the antidepressant-like effects in the learned helplessness model.

It has been recently proposed that ketamine exerts its antidepressant effect through the influence of its hydroxynorketamine (HNK) metabolite (2R,6R)-HNK (Zanos et al., 2016). *In vivo*, racemic ketamine is rapidly metabolized to several stereoisomers of HNK and norketamine. A 10 mg/kg (i.p.) administration of (2R,6R)-HNK exerted an antidepressant-like response in mouse FST and learned helplessness. However, a modified form of ketamine, deuterated in the C6-position so that its metabolism to HNK is hindered while its pharmacological properties are maintained, lacked any antidepressant-like effects. The behavioral effects of (2R,6R)-HNK were also blocked by a pretreatment with NBQX, demonstrating the effects to be dependent on AMPAR activation. Moreover, (2R,6R)-HNK lacked ketamine-related drug-seeking behavior and side effects, such as deficits in prepulse inhibition and locomotor coordination in rotarod test. Further studies indicated (2R,6R)-HNK to elicit an antidepressant-like response through negative modulation of presynaptic metabotropic glutamate receptor 2 ($mGluR_2$) autoreceptors that under basal conditions inhibit presynaptic glutamate release (**Figure 4D**) (Zanos et al., 2019). Other studies, however, found no effects of HNK on depressive-like behavior in mice subjected to lipopolysaccharide injections or chronic social defeat stress (Yamaguchi et al., 2018; Yang et al., 2017) or in rats subjected to learned helplessness (Shirayama & Hashimoto, 2018). Furthermore, small-scale clinical studies have found an inverse correlation between plasma HNK concentration and antidepressant response after ketamine infusions (Farmer et al., 2020; Grunebaum et al., 2019). Therefore, the role of HNK in ketamine's antidepressant effects remains controversial.

In addition to NMDARs, ketamine targets several other receptors and neurotransmitter systems in the brain, some of which may contribute to its antidepressant effects. For instance, a potential role for the opioid system in ketamine's action has been indicated since pretreatment with opioid receptor antagonist naltrexone negated ketamine's antidepressant effect in a small-scale clinical study (Williams et al., 2018). In addition, ketamine's antidepressant-like effects in FST were abolished by the dopaminergic D2/D3 receptor antagonist haloperidol, and by central depletion of serotonin, indicating a role for monoaminergic systems as well (Gigliucci et al., 2013; Li et al., 2015). Other identified targets of ketamine include, among others, muscarinic and nicotinic acetylcholine receptors and voltage-gated ion channels, as reviewed in Zanos et al. 2018 (**Table 1**).

Table 1. Identified receptor targets of (R, S)-ketamine. HCN1 = hyperpolarization-activated cyclic nucleotide-gated potassium channel 1. Adopted from Zanos et al., 2018.

Target receptor	Action
NMDAR	Antagonist
HCN1	Inhibitor
$GABA_AR$	Positive modulator
Muscarinic Acetylcholine receptor	Antagonist
Nicotinic Acetylcholine receptor	Antagonist
D2/D3 receptor	Partial agonist
Dopamine transporter	Inhibitor
$5\text{-}HT_3R$	Antagonist
Serotonin transporter	Inhibitor
Noradrenaline transporter	Inhibitor
μ opioid receptor	Agonist
$\varkappa$ opioid receptor	Agonist
δ opioid receptor	Agonist
Voltage-gated sodium channel	Antagonist
L-type Voltage-dependent calcium channel	Antagonist

Overall, various hypotheses have been proposed to underlie ketamine's antidepressant effects, but shared components of the proposals include increased glutamatergic tone and AMPAR-dependent regulation of neurotrophic signaling and a subsequent synthesis of synaptic proteins that leads to alterations in synaptic plasticity (**Figure 4**). Further insight into the precise antidepressant mechanisms of ketamine may be gained by considering the similar effects that other somatic and pharmacological rapid-acting antidepressants induce in the brain.

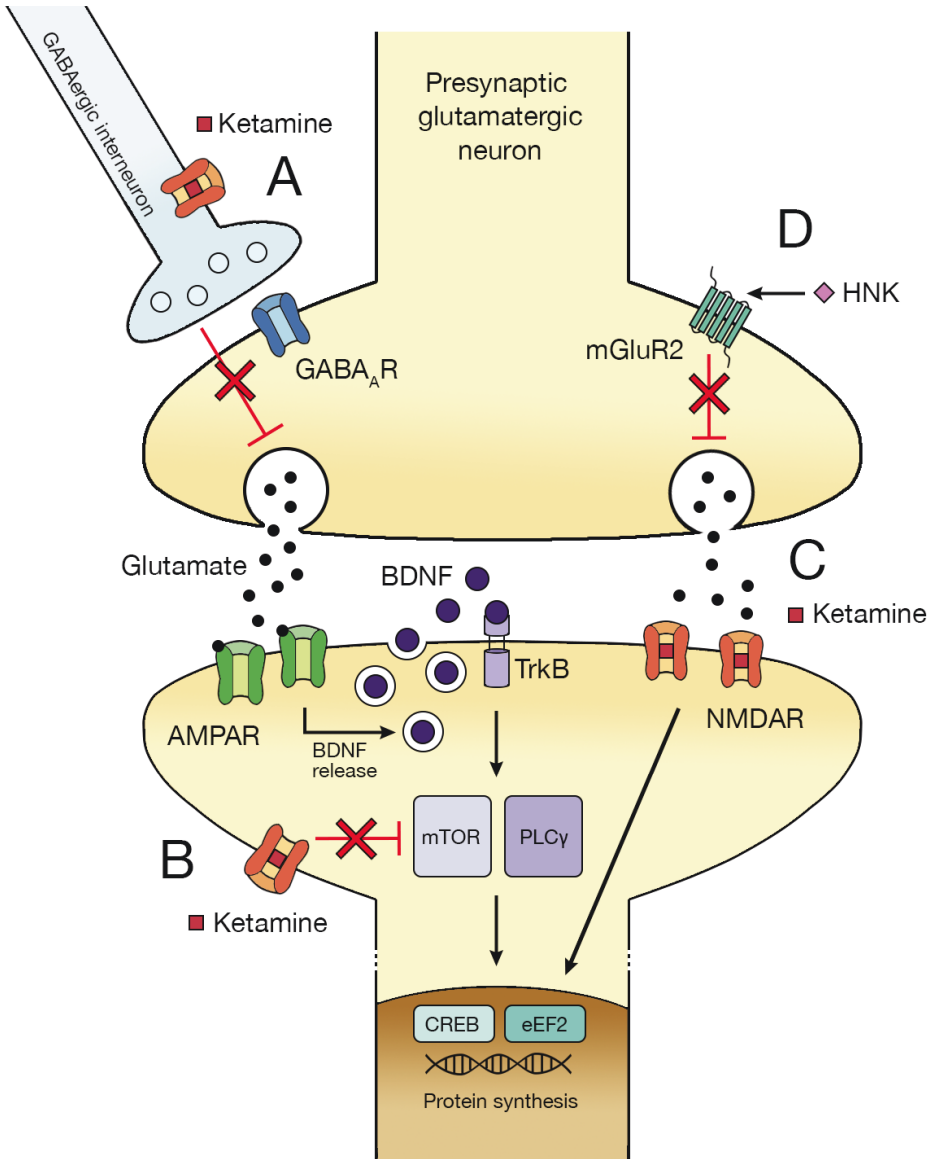

Figure 4. Proposed cellular mechanisms of ketamine's antidepressant action. Suggested mechanisms of ketamine include A) disinhibition of pyramidal neurons through selective blockade of NMDARs in GABAergic interneurons, B) blockade of extrasynaptic NMDARs that disinhibits mTOR activity, C) blockade of spontaneous NMDAR activation that dephosphorylates eEF2, allowing for rapid translation of BDNF, D) disinhibition of presynaptic glutamate release through blockade of $mGluR_2$ by HNK. Different proposals converge on BDNF-dependent upregulation of synaptic protein synthesis and increased synaptogenesis. Figure modified after Zanos & Gould (2018).

2.5. Shared mechanisms of rapid-acting treatment options

Despite the increasing enthusiasm for the antidepressant effects of ketamine, it is by no means the first treatment option that has demonstrated rapid antidepressant effects. Indeed, ECT has long been the treatment of choice when classical antidepressants have failed to produce treatment response or a more rapid alleviation of symptoms is critical due to acute suicidality (Payne & Prudic, 2009). Interestingly, sleep deprivation has also consistently shown to rapidly alleviate depressive symptoms in patients, although the effect is usually short-lived (Giedke & Schwärzler, 2002; Wu & Bunney, 1990). Moreover, a variety of pharmacological agents demonstrating rapid antidepressant efficacy have emerged. Many of these are anesthetics, but classical psychedelics, such as psilocybin, have also gained recent research interest (Carhart-Harris & Goodwin, 2017). All of these treatments modulate cortical excitation-inhibition balance and neuronal excitability, but neurotrophic mechanisms have also been implicated, indicating that parallel neurobiological processes may underlie the antidepressant effects of these diverse rapid-acting treatment approaches.

ECT was first introduced in the treatment of psychiatric disorders in the 1930s (Payne & Prudic, 2009). Despite decades of drug development, ECT remains among the most effective antidepressant treatments in clinical use (Pagnin et al., 2004). It is generally considered to be a "last resort" treatment, when the patient has failed to respond to several conventional antidepressant treatments, or under clinical urgency, when the patient is expressing hallucinations or delusions, catatonia, or suicidality (Payne & Prudic, 2009). Other indications of ECT include schizophrenia, catatonia, and manic bipolar states (Kellner et al., 2012). In ECT, a brief pulse of electrical current is delivered to the anesthetized patient's scalp, leading to transient epileptiform activity in the EEG. This treatment can efficiently and rapidly alleviate depressive symptoms even when conventional antidepressants have failed to do so (Abbott et al., 2014; Pagnin et al., 2004; UK ECT Review Group, 2003). Typically, repeated stimulations are required to achieve therapeutic remission, but the response to ECT usually emerges faster than the response to prescription antidepressants (Pagnin et al., 2004). There are currently no clearly identified clinical predictors for ECT response. However, recent meta-analyses have found ECT to be particularly effective in elderly people and patients with psychotic symptoms, short depressive episodes, or no previous medication failures (Haq et al., 2015; Van Diermen et al., 2018). In addition, a specific gene promoter methylation of p11 has been proposed as a biomarker to predict ECT treatment response (Neyazi et al., 2018).

Despite a negative impression of ECT among general public, fueled, among other things, by its inaccurate depiction in popular culture, modern ECT involves oxygenation, general anesthesia with neuromuscular blockade, and physiological monitoring, making it a relatively safe treatment option (Husain et al., 2004). Adverse effects of ECT include transient anterograde amnesia, head and muscle ache, and nausea, but these effects often pass within hours of treatment (Lisanby, 2007). A rarer but persistent adverse effect is retrograde amnesia,

which commonly involves poor recollection of events directly before ECT administration but sometimes includes amnesia for events dating months or even years before ECT.

Neurobiological mechanisms underlying the antidepressant action of ECT remain poorly understood, but various proposals have suggested seizure activity, anticonvulsant effects, and neurotrophic function to be involved (Abbott et al., 2014). The importance of seizure activity was established in the 1960s, when pretreatment with an anticonvulsant drug, lidocaine, was found to abolish the antidepressant effects of ECT (Cronholm & Ottosson, 1960). The intriguing ability of seizures to rapidly alleviate depressive symptoms is further supported by findings regarding a volatile convulsant, flurothyl (hexafluorodiethyl ether) (Fink, 2014). A seizure induced by flurothyl inhalation was found to be equally efficient to ECT in the treatment of depression but with lower incidence of cognitive side effects (Small et al., 1968). However, it has been acknowledged that generalized seizures per se do not reliably produce antidepressant effects, indicating that other factors must be involved in the antidepressant effects of ECT (Perera et al., 2004). The anticonvulsant hypothesis is based on observations indicating that the ECT-induced increase in seizure threshold correlates with the treatment's antidepressant response (Sackeim, 1999). Localization of the stimulus and delivered electrical dosage have also been shown to play a role, since remission rates and cognitive side effects of ECT have been demonstrated to depend on the electrode placement and stimulus intensity (Lisanby, 2007; Nobler et al., 1993; Sackeim et al., 2000).

ECT has been shown to influence a variety of brain functions, including monoaminergic, GABAergic, and glutamatergic neurotransmission, neuroendocrine function, and neurotrophic signaling (Kellner et al., 2012). The neurotrophic effects in particular are reminiscent of those induced by ketamine and other antidepressants. In depressed human patients, plasma BDNF levels normalized to the levels of healthy controls after ECT (Piccinni et al., 2009). This effect correlated with treatment response since non-responders demonstrated a more modest increase in plasma BDNF levels. Repeated electroconvulsive seizures (ECS, an animal model of ECT) influenced neurotrophic action in the brain by increasing the expression of BDNF mRNA (Altar et al., 2004; Nibuya et al., 1995; Smith et al., 1997; Zetterström et al., 1998) and protein (Altar et al., 2003; Angelucci et al., 2002) in the rat PFC and HC. ECS in rodents has also been shown to upregulate various molecular markers connected to neuronal excitation. It acutely upregulated several activity-dependent immediate-early genes (IEGs), including *Arc*, *c-fos* and *Egr1* in hippocampal and cortical regions in rodents (Dyrvig et al., 2014; Larsen et al., 2005). IEGs play a significant role in the genomic response to stimulus as their products include transcription factors and effector proteins that are involved in synaptic modifications and plasticity (Dyrvig et al., 2014). In addition, a single ECS caused a robust upregulation of MAPK phosphorylation in the rat PFC (Hansen et al., 2007). Unexpectedly, ECS acutely decreased TrkB phosphorylation.

Increased neurogenesis and synaptogenesis have also been implicated in ECT's antidepressant actions. ECS increased neurogenesis in the rat dentate gyrus (Madsen et al., 2000) and cell

proliferation in the frontal cortex (Madsen et al., 2005). Another study found repeated ECS treatments to increase hippocampal volume and the number of hippocampal neurons and spine synapses (Chen et al., 2009). Similar observations have also been made in non-human primates, where three ECS administrations per week for four weeks induced cell proliferation and neurogenesis in the male bonnet monkey dentate gyrus (Perera et al., 2007). The effects were observed immediately after the treatment course and were sustained at four weeks after the interventions.

EEG has been widely used as a research tool for ECT since its entry into the clinical domain (Nobler et al., 1993; Sackeim, 1999). Initially, seizure duration was believed to predict the antidepressant efficacy of ECT, but this hypothesis was later discarded. More recently, the search for predictive treatment efficacy markers of ECT has been focused on longitudinal EEG signature during and after treatment. In particular, the antidepressant efficacy of ECT has been linked to postictal suppression of cortical EEG activity (Nobler et al., 1993; Sackeim et al., 1996; Suppes et al., 1996). This electrocerebral silence emerged soon after the seizure and was associated with a widespread decrease in cerebral blood flow (Nobler et al., 1994) and metabolism (Nobler et al., 2001). Increased postictal delta EEG power (1–4 Hz) was associated with a positive treatment response at 2-month follow-up monitoring (Sackeim et al., 1996). No notable postictal slowing was observed with ineffective low intensity right unilateral ECT (Sackeim et al., 2000). This determinant of treatment outcome also encouraged the initial studies examining the antidepressant effects of isoflurane anesthesia since it produced a similar slowing of cortical EEG activity (Langer et al., 1985, 1995).

Indeed, deep isoflurane anesthesia also rapidly regulated molecular alterations intimately associated with the therapeutic effects of ketamine, namely TrkB signaling (Antila et al., 2017). The effects of isoflurane on TrkB were dose-dependent, and the most prominent effects were seen with a dosing regimen leading to a burst-suppressing EEG pattern (Theilmann et al., 2019). Notably, isoflurane regulated TrkB signaling far more readily than ECS (Antila et al., 2017; Hansen et al., 2007). A brief exposure to isoflurane anesthesia produced antidepressant-like behavioral responses in the rat learned helplessness model of depression (Brown et al., 2018a). Interestingly, the same study found another anesthetic compound, halothane, which lacks the capability to produce isoelectric EEG and burst-suppression, to bring no such effects although it readily regulates TrkB signaling (Antila et al., 2017; Brown et al., 2018a).

Another anesthetic that has demonstrated antidepressant potential is nitrous oxide (N_2O), commonly referred to as “laughing gas.” N_2O is a colorless and odorless gas with over 150 years of history in clinical use (Sanders et al., 2008). Like ketamine, the main pharmacological function of N_2O is NMDAR antagonism. N_2O is primarily used in clinical anesthesia and analgesia in labor and delivery, and dentistry. It is one of the least potent anesthetics, with a minimum alveolar concentration (MAC: alveolar concentration of inhaled anesthetic that prevents motor response in 50% of patients in response to surgical incision) of 104%, whereas isoflurane, for instance, has a MAC value of 1.17% (Mapleson, 1996). This means that N_2O

by itself is insufficient in producing surgical anesthesia since, to avoid hypoxia, it can be administered only at a maximum concentration of 70% (Nagele et al., 2018). It is therefore mainly used as an adjunct agent in combination with other anesthetics. N_2O has a notably rapid onset and offset of action, and it does not undergo significant metabolism. In a recent clinical study, N_2O demonstrated rapid antidepressant efficacy in treatment-resistant patients (Nagele et al., 2015). Similarly to the effects of ketamine, the antidepressant effects of N_2O were most pronounced 24 hours after the treatment and lasted up to a week in some patients. However, N_2O also demonstrates misuse potential, reported as long ago as the Victorian era, since it is recreationally used to achieve brief euphoric intoxication as inhalations from whipped cream containers (van Amsterdam et al., 2015). Studies examining the neurobiological effects of N_2O in an antidepressant context have so far remained scarce.

In addition to ECT, sleep deprivation provides a non-pharmacological means to alleviate depressive symptoms. Sleep deprivation for 24–48 hours has been robustly shown to relieve depression in a subset of patients (Wu & Bunney, 1990). The effects, however, remain transient, and a depressive relapse commonly occurs after a subsequent sleep period (Wiegand et al., 1993; Wu & Bunney, 1990). However, for some patients, the improvement of symptoms can last up to weeks (Giedke & Schwärzler, 2002). These findings are supported by animal studies demonstrating increased swimming behavior of rats in FST after 24 hours of sleep deprivation (Lopez-Rodriguez et al., 2004). Interestingly, sleep deprivation also shares several molecular effects with other rapid-acting antidepressant treatments. Total and partial sleep deprivation for one night has been reported to rapidly elevate plasma BDNF levels for the following day in depressed patients (Giese et al., 2014; Gorgulu & Caliyurt, 2009). Moreover, the increase in plasma BDNF levels was found to correlate with the antidepressant treatment response (Gorgulu & Caliyurt, 2009). Another study found a 24-hour sleep deprivation in naïve rats to increase *Bdnf* mRNA levels, particularly in hippocampal regions (Conti et al., 2007). Sleep deprivation also upregulates IEG *c-fos* in several areas of the rat brain (Cirelli et al., 1995). Functional neuroimaging studies have shown sleep deprivation responders to express increased metabolic activity in the medial PFC and ventral anterior cingulate cortex (Wu et al., 2001, 2008). To our knowledge, the effects of sleep deprivation on TrkB signaling have not been investigated.

Another putative class of compounds that has demonstrated rapid antidepressant effects is classical psychedelics, which includes psilocybin and lysergic acid diethylamide (LSD) (Carhart-Harris & Goodwin, 2017). Classical psychedelics have a pharmacological mechanism distinct from that of anesthetics in that they primarily function as agonists in serotonergic 5-HT_{2A} receptors (Nichols, 2016). After decades of hiatus in the clinical research of psychedelics, recent findings have revitalized interest in the study of their potential for treating mood disorders. In a preliminary clinical trial, a single dose of psilocybin demonstrated rapid antidepressant effects that were sustained even 3 months after the treatment (Carhart-Harris et al., 2016). *In vitro*, LSD and other serotonergic psychedelics have been demonstrated to increase dendritic spine density and induce synaptogenesis similarly to

ketamine (Ly et al., 2018). These effects were blocked by TrkB inhibitor ANA-12, indicating that these effects are mediated by TrkB activation. However, these neurotrophic effects have yet to be investigated *in vivo*.

In conclusion, multiple complex mechanisms appear to be involved in a rapid-acting antidepressant response, but alterations in cortical excitation and neurotrophic action and the subsequent recruitment of cellular machinery that leads to morphological and functional synaptic plasticity has been implicated in nearly every effective antidepressant treatment. However, these findings have not yet translated to clinical breakthroughs, indicating unresolved questions about the role of neurotrophic signaling in a rapid antidepressant response.

3. Aims of the study

Anesthetics profoundly impact neuronal function during different stages of development, and they have garnered academic interest in the treatment of MDD. Burst-suppressing anesthesia and subanesthetic ketamine, in particular, have been shown to rapidly alleviate depressive symptoms in adults, but the underlying neurobiological mechanisms remain debated. Moreover, the clinical results of burst-suppressing anesthesia have so far been inconsistent. Despite having a pharmacological target clearly distinct from that of ketamine, isoflurane was recently shown to induce neurotrophic signaling, a key mechanism implied in the rapid antidepressant effects of ketamine (Antila et al., 2017). The main objectives of this thesis are to further elucidate the behavioral effects of isoflurane in rodents with two approaches—by determining the potential adverse effects of early-life anesthesia and testing its efficacy in a rodent model of depression—and to investigate how different antidepressant anesthetics regulate molecular and electrophysiological events associated with the rapid antidepressant effects of ketamine.

The specific aims of the subprojects in this thesis were as follows.

I Investigate the long-term behavioral consequences of repeated brief isoflurane anesthesia when delivered during early postnatal development in male mice (I).

II Investigate the antidepressant effects of deep burst-suppressing isoflurane anesthesia in a rat chronic mild stress model of depression (II).

III Investigate the shared neurobiological effects evoked by rapid-acting antidepressants ketamine, nitrous oxide, and flurothyl in adult mice (III, IV) by:

- conducting pharmaco-EEG studies in freely-moving adult mice
- studying time-dependent effects on key molecular determinants associated with rapid antidepressant effects
- studying dose- and time-dependent effects of ketamine (and its hydroxynorketamine metabolite) on EEG and neurotrophic signaling.

4. Materials and methods

The main methods used in this thesis are briefly outlined here. The details of the methods and specifications of the chemicals and consumables used are described in corresponding research articles or their supplements.

4.1. Animals

Studies I, III, and IV were conducted using C57BL/6JRccHsd mice (Harlan Laboratories/Envigo, Venray, The Netherlands). Animals were maintained in standard conditions (21 °C, 12-h light-dark cycle) in the facilities of the University of Helsinki with *ad libitum* access to food and water.

In study II, we used a Crl:WI(Han) substrain of Wistar outbred rats (Charles River Laboratories, Wilmington, Massachusetts, USA) since this strain was previously shown to be particularly prone to attaining a depressive-like phenotype in response to CMS (Theilmann et al., 2016).

All the animal experiments were carried out according to the guidelines of the Society for Neuroscience and were approved by the relevant national authorities.

4.2. Behavioral analysis

In study I, to achieve a wide phenotypic characterization of adult mice, the animals were exposed to a battery of behavioral tests that included home-cage activity measurement, evaluation of nesting behavior, saccharin preference test, measurement of stress-induced hyperthermia, light-dark box test, open field test, prepulse inhibition test, Morris water maze test, and FST.

In study II, anhedonic-like and anxious behavior was measured by sucrose consumption test, open field test, and EPM test. Sucrose consumption test is a canonical method of measuring hedonic deficits in rodents (Willner et al., 1987). Animals had access to 1% sucrose solution in addition to normal drinking water, and their overnight consumption of sucrose solution was measured.

In study III, a well-established learned helplessness model was used to model depressive-like behavior. In learned helplessness, the rodent is exposed to inescapable foot shocks (Seligman et al., 1975). When later placed in a chamber where escape from the shocks is possible, an animal with learned helplessness fails to escape, whereas an unstressed animal would. Learned helplessness behavior is alleviated by several antidepressant drugs.

4.3. Chronic mild stress

In rodents, CMS is a widely accepted model of depression since it recapitulates a set of depressive-like symptoms, such as anhedonia. In rodents, a decreased preference for the consumption of sweet solution is considered anhedonic behavior (Willner et al., 1987). In study II, we used a stress-sensitive substrain of rats (Crl:WI[Han]) previously shown to respond to ECS but not the SSRI drug citalopram (Neyazi et al., 2018). We used a CMS protocol that was derived from the protocol described by Willner et al. (1987). Briefly, the animals were exposed to one randomized mild stressor every day for three weeks. The stressors included swimming in cold (15°C) or hot (40°C) water for 5 or 10 minutes, respectively, wet bedding for 16 hours, food deprivation for 24 hours, water deprivation for 14–21 hours, restraint stress for 30–60 minutes, and continuous light for 36 hours.

4.4. Sample collection and preparation

In studies II–IV, brain samples for the biochemical analyses were collected at time points indicated in the research articles. Briefly, animals were euthanized by cervical dislocation, decapitated, and the brain was rapidly collected and submerged in ice-cold phosphate-buffered saline. Bilateral PFC and HC samples were manually dissected on a cold plate, snap frozen on dry ice, and stored at -80°C until further processing. For total protein extractions, the brain samples were homogenized in ice-cold lysis buffer. The samples were centrifuged, and the resulting supernatant was collected for further analysis. For quantitative polymerase chain reaction (qPCR), total ribonucleic acid (RNA) was extracted from the samples by homogenization in Trizol reagent (ThermoScientific). RNA was then phase separated by incubating the homogenized samples in chloroform. The aquatic phase containing the RNA was collected, and the RNA was precipitated with isopropanol, washed twice with 75% ethanol, and dissolved in nuclease-free water.

4.5. Biochemical analysis of samples (Western blot, PCR, ELISA)

Changes in protein phosphorylation were assayed by Western blotting as described (Kohtala et al., 2016). Briefly, homogenized PFC sample lysates were heated in Laemmli buffer at +100°C for 3 minutes, separated in SDS-PAGE under reducing conditions, and blotted on a PVDF membrane. Membranes were incubated overnight with the antibodies reported in the original research articles, followed by washes with tris-buffered saline with 0.1% Tween 20 and a 1-hour incubation with horseradish peroxidase-conjugated secondary antibody (1:10 000 in non-fat dry milk). After subsequent washes, the membranes were visualized by enhanced chemiluminescence (ECL Plus, ThermoScientific) and detected with a Biorad ChemiDoc MP camera (Bio-Rad Laboratories).

For qPCR, the RNA extracts were treated with DNAse to digest residual DNA, and the mRNA was subsequently reverse transcribed to complimentary DNA (cDNA) with oligo (dT) primer and SuperScript III Reverse Transcriptase Mix (ThermoScientific). Real-time qPCR was used to quantify the cDNA expression in the reverse-transcribed PFC samples. Briefly, specific cDNA regions were amplified using the primers reported in the original articles. Amplification reactions were run in LightCycler 480 (Roche) in the presence of SYBR Green reagent (ThermoScientific).

In study II, BDNF protein levels were detected via BDNF-specific enzyme-linked immunosorbent assay (ELISA). The protein extracts were acidified to pH 3 by 1 M HCl, incubated for 15 minutes, and neutralized with 1 M NaOH. The samples were loaded onto a 96-well, pre-coated with monoclonal BDNF antibody, pre-blocked with bovine serum albumin, and incubated for 2 hours. HRP-conjugated secondary monoclonal BDNF antibody was added on the plate and, after a subsequent 1-hour incubation and washes, incubated with color reagents (hydrogen peroxide and chromogen) for 30 minutes. The reaction was stopped with 2 M sulphuric acid, and the absorbance of the wells in 450 nm was measured.

4.6. EEG

In studies III and IV, EEG was recorded via screws surgically placed above the fronto-parietal cortex of the mice. Electromyograph (EMG) was also recorded from silver wires placed in the nuchal muscles to monitor the vigilance state of the rodents. During the surgery, animals were anesthetized with isoflurane (3% induction, 1.5–2% maintenance). Lidocaine (10%) was used for local anesthesia and buprenorphine (0.1 mg/kg, s.c.) for post-operative care. All EEG recordings and data processing were performed using Spike2 software (version 8.07, Cambridge Electronic Devices).

5. Results

5.1. Long-term behavioral effects of repeated isoflurane anesthesia during early development (I)

Anesthesia during early brain development has been associated with neuropathological changes in rodents, including neuroapoptosis, synapse loss, and persistent behavioral abnormalities (Briner et al., 2010; Jevtovic-Todorovic et al., 2003). However, the role of the depth, duration, and developmental time point of exposure to anesthesia remains unclear. We exposed mouse pups to three brief sessions of isoflurane anesthesia (4% induction, 2% maintenance, 30 min) for three consecutive days in two cohorts: at either postnatal days (P)7–9 or P15–17 (**Figure 5**). These two time points were selected because earlier studies have shown anesthetics to have different effects on the developing brain at these points. While anesthesia exposure at P7–9 has been consistently associated with apoptotic neurodegeneration (Jevtovic-Todorovic et al., 2003; Tagawa et al., 2014), a later administration at P16 has been shown to increase dendritic spine density (Briner et al., 2010; De Roo et al., 2009). Our aim was to determine whether these differential neurobiological effects would be reflected in different behavioral outcomes in adult animals. Since the pups had to be separated from their dams for the duration of anesthesia, another group of animals from both cohorts were subjected to maternal separations (MS) of similar duration to control the potential detrimental effects of MS. The animals were weaned at P21, and, beginning from postnatal week 9, the mouse phenotype was evaluated using a battery of behavioral tests that assess learning and memory, general locomotor activity, and depression, anxiety, and schizophrenia-related endophenotypes.

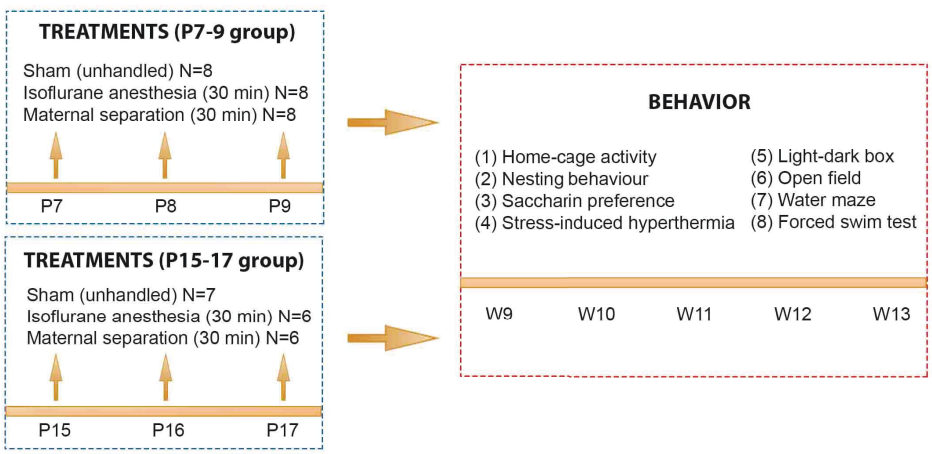

Figure 5. Experimental timeline. Abbreviations: P = postnatal day; W = postnatal week. Figure reprinted and modified from publication I.

When isoflurane anesthesia was delivered at P7–9, the treatments caused no gross phenotypic alterations in adult behavior. Animals subjected to anesthesia demonstrated only subtle deficits in spatial learning and memory in the Morris water maze test (**Figure 6**). However, similar deficits were also present in the maternal separation group; thus, the effects cannot be concluded to arise specifically from isoflurane administrations.

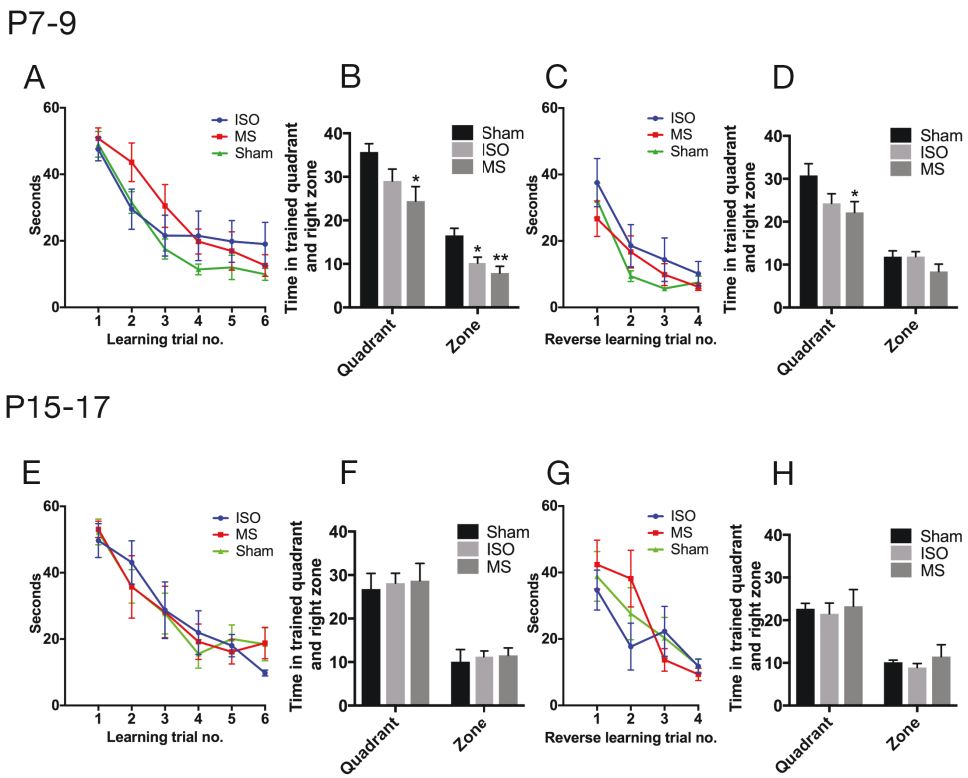

Figure 6. Early-life exposure to three consecutive and brief sessions of isoflurane anesthesia or maternal separations at postnatal days 7–9 bring mild deficit in spatial navigation memory. Latency to escape (find the platform) (**A, E**) and time spent near the vicinity of pre-existing platform (quadrant, zone) (**B, F**) during the first learning trials and probe test, respectively. Latency to escape (find the platform) (**C, G**) and time spent near the vicinity of pre-existing platform (quadrant, zone) (**D, H**) during the reverse learning trials and probe test, respectively. Abbreviations: ISO = isoflurane; MS = maternal separation. Data are presented as mean ± SEM. *< 0.05, **< 0.01, two-way ANOVA followed by Newman-Keuls *post hoc* test. Figure reprinted and modified from publication I.

In the P15–17 cohort, the most pronounced behavioral change was observed in the home cage motor activity monitoring. Curiously, isoflurane and maternal separation produced contrasting differences in the general locomotor activity of adult animals, with isoflurane-treated animals showing hyperactive behavior in relation to non-treated animals, and the MS group showing decreased locomotor activity (**Figure 7**). These effects were particularly pronounced during the active period (dark phase) of the animals.

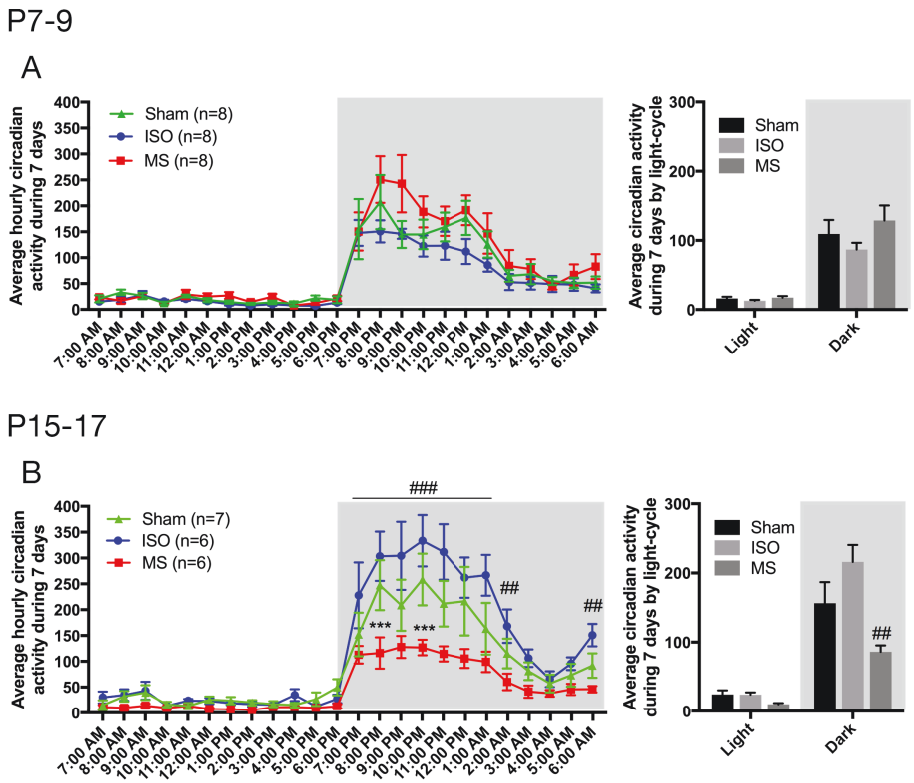

Figure 7. Animals exposed to repeated brief isoflurane anesthesia or maternal separation at postnatal days 15–17 show varying circadian activity at adult age. Hourly average circadian activity during 7-day monitoring and average circadian activity during different light-cycles. Lights off (active period; gray) during 6:00 PM–6:00 AM. Abbreviations: ISO = isoflurane; MS = maternal separation. Data are presented as mean ± SEM. ***< 0.001 two-way ANOVA followed by Newman-Keuls *post hoc* test MS vs. Sham. ###< 0.001, ##< 0.01 two-way ANOVA followed by Newman-Keuls *post hoc* test ISO vs. MS. Figure reprinted and modified from publication I.

Overall, our study indicates that repeated brief exposure to isoflurane anesthesia during early postnatal development in mice produces differential, yet relatively modest behavioral alterations in adulthood, that depend on the developmental time point of administration.

5.2. Lack of behavioral effects of isoflurane anesthesia in the rat CMS model (II)

Postictal slowing of EEG activity has been proposed to predict the onset of action of the antidepressant effects of ECT (Nobler et al., 1993; Sackeim et al., 1996; Suppes et al., 1996). Isoflurane has a capability to produce electrocerebral silence similar to that of ECT during anesthesia but without the preceding convulsions and seizure activity. Indeed, repeated isoflurane anesthesia has demonstrated intriguing antidepressant potential in preliminary clinical studies (Langer et al., 1985, 1995; Weeks et al., 2013). Moreover, only a single, brief isoflurane anesthesia has demonstrated antidepressant-like effects in the FST, learned helplessness model, and CMS model in mice (Antila et al., 2017; Brown et al., 2018a; Zhang et al., 2019). To test this "cerebral silence" hypothesis and further elucidate the antidepressant potential of isoflurane, we used the CMS model in a substrain of rats that have been previously shown to respond to ECS but not to the SSRI antidepressant citalopram (Neyazi et al., 2018).

After 3 weeks of CMS exposure, the stress-exposed rats were divided into stress-resilient and anhedonic groups, based on the individual rats' response to stress in the sucrose consumption test. Rats whose sucrose consumption showed a >25% within-subject decrease were considered anhedonic (14 out of 26 stressed animals), while those that showed <10% decrease in sucrose consumption were considered stress-resilient (12 out of 26 animals). After the stress exposure, animals were exposed to repeated sessions of isoflurane anesthesia (4% induction for 2 minutes, 2% maintenance for 13 minutes) or sham treatments in an anesthesia chamber (room air, 15 minutes) for a total of five times, once every three days. Anhedonic-like and anxious behavior was measured after the first, third, and fifth treatments in a sucrose consumption test and open field test, and after the fifth treatment in an EPM test (**Figure 8**).

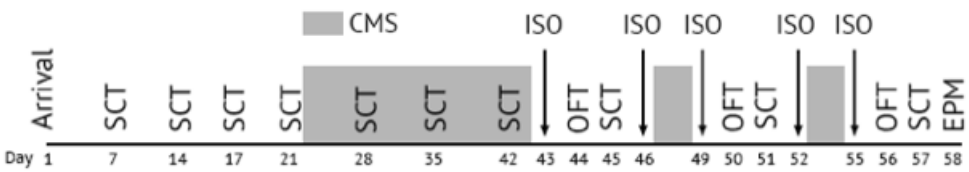

Figure 8. Experimental timeline. CMS = chronic mild stress; EPM = elevated plus-maze; ISO = isoflurane; OFT = open field test; SCT = sucrose consumption test. Figure reprinted from publication II.

Somewhat unexpectedly, isoflurane had essentially no impact on the behavior of the stressed rats at any point during the course of experiments (**Figure 9**). It is unlikely that this lack of isoflurane effect is due to insufficient "EEG silencing" since the employed dosing generates noticeably reproducible EEG burst-suppression (Leikas et al., 2017; Theilmann et al., 2019).

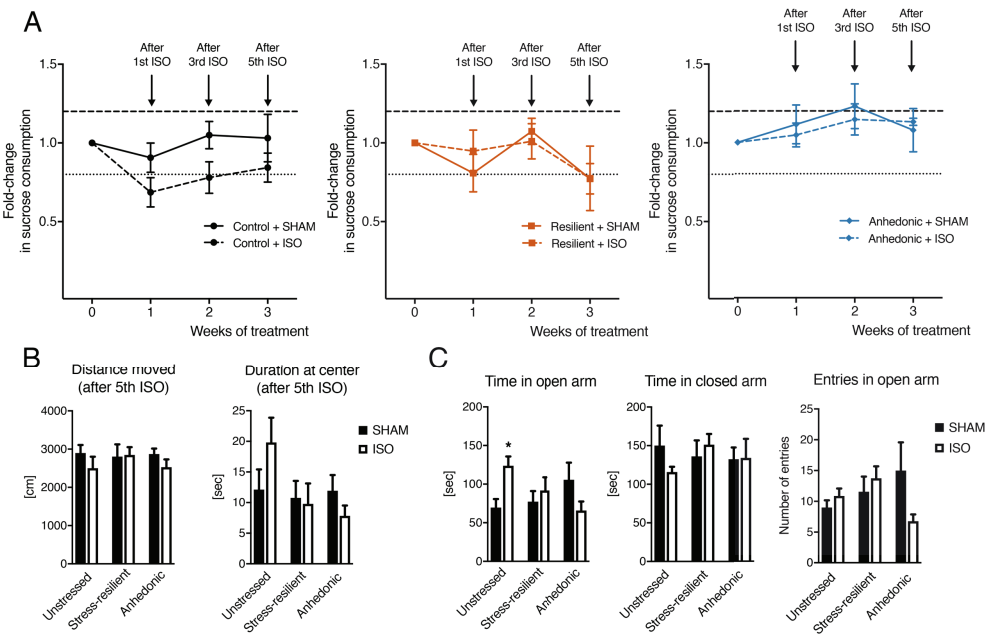

Figure 9. Repeated isoflurane exposure caused no notable behavioral alterations in any of the treatment groups. (**A**) Changes in sucrose consumption in different treatment groups over the course of three weeks. (**B**) Distance moved and duration spent at the center of the arena in open field test after the 5th isoflurane exposure. (**C**) Effects of repeated exposure to isoflurane anesthesia in elevated plus maze test. Data are presented as mean ± SEM. *< 0.05, two-way ANOVA followed by Sidak's multiple comparisons test. Figure reprinted from publication II.

Another goal of our study was to examine whether the depressive-like phenotype after CMS would be reflected in cortical and hippocampal BDNF expression and whether the treatment with isoflurane would renormalize it. Stress and depression have been connected to decreased expression of BDNF (Smith et al., 1995). Furthermore, elevation in hippocampal BDNF mRNA levels have been consistently reported after ECS (Nibuya et al., 1995; Smith et al., 1997). In line with behavioral observations, we observed no differences in BDNF expression in PFC or HC between treatment groups or endophenotypes, as measured by ELISA (**Figure 10**). Interestingly, our administration protocol consistently induced cortical TrkB signaling (Antila et al., 2017; Theilmann et al., 2019).

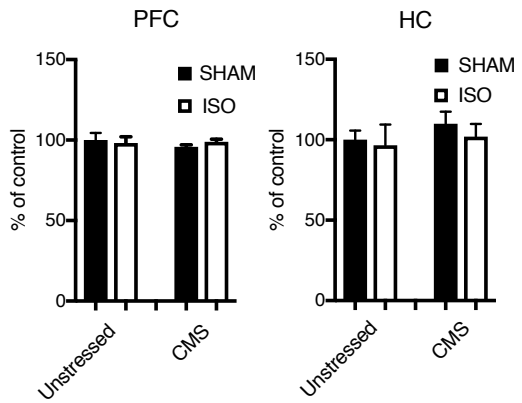

Figure 10. BDNF protein levels remained unaltered in PFC and HC in response to CMS and repeated isoflurane treatments. Data are presented as mean ± SEM. Figure reprinted and modified from publication II.

5.3. Nitrous oxide evokes TrkB signaling and slow-wave EEG activity after its acute pharmacological effects have subsided (III)

Like ketamine, N_2O is an NMDAR antagonist that has been shown to alleviate depressive symptoms in a subset of patients (Nagele et al., 2015). In study III, we wanted to determine the effects of N_2O on biochemical and electrophysiological markers indicated in the rapid antidepressant effects of ketamine. Our specific focus in the biochemical experiments was on TrkB signaling and activity-dependent IEGs implicated in antidepressant effects. We took advantage of the fast pharmacokinetic properties of N_2O, which allowed us to investigate the temporal regulation of these effects both during and after the acute drug-induced NMDAR blockade. The period after the acute effects is particularly interesting, since the antidepressant effects of ketamine are known to emerge preferentially after the psychotomimetic effects subside (Zarate et al., 2006a).

To test whether subanesthetic doses of ketamine and N_2O share the ability to induce EEG slow-wave activity (SWA) similarly to ECT, we measured the effects of these treatments on mouse EEG across the drugs' acute effects and withdrawal. As previously reported, a subanesthetic dose of ketamine (10 mg/kg, i.p.) caused an acute increase in EEG gamma oscillations (25–100 Hz) for approximately 30–50 minutes (**Figure 11A**) (Hiyoshi et al., 2014). After these acute effects subsided, we observed a gradual increase of slow-wave delta oscillations (1–4 Hz) that exceeded those observed in the saline-treated control group. With N_2O (50% in O_2 for 1 h), we discovered a general dampening of EEG power for the whole duration of treatment (**Figure 11B**). After terminating the gas flow, however, we observed a gradual increase of slow-wave delta oscillations, similar to that observed with ketamine, well above baseline levels.

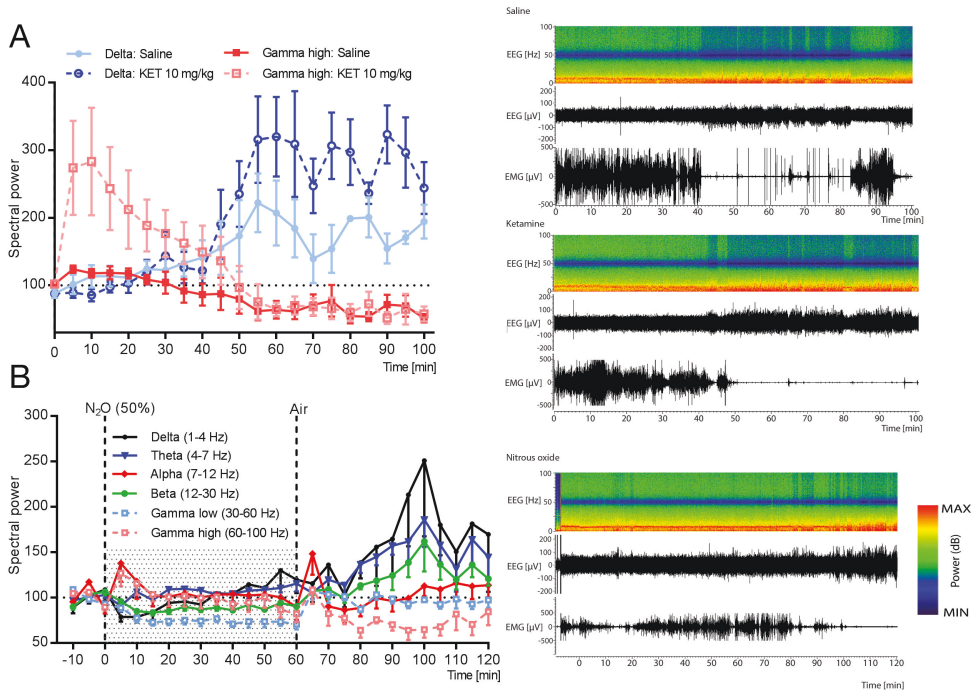

Figure 11. Ketamine and nitrous oxide cause a "rebound" increase in delta EEG power after acute pharmacological effects subside. Normalized EEG power of main oscillations and representative time-frequency spectrogram after (**A**) ketamine (10 mg/kg, i.p., 100 min) and (**B**) N_2O (50% in O_2, 60 min + 60 min washout) administration. Data are presented as mean ± SEM. Figure reprinted and modified from publication III.

Next, we wanted to examine whether N_2O induces rapid cortical excitation. We adopted the treatment protocol from the proof-of-concept clinical study by Nagele et al. (2015) and exposed animals to 50% N_2O for one hour in an anesthesia chamber. The animals were allowed a one-hour recovery in their home cage before collection of PFC samples. Expressions of activity-dependent IEGs were assayed with quantitative real-time PCR. The levels of IEG (e.g., *Arc*, *Bdnf*, *c-fos*) mRNAs were upregulated after N_2O exposure (**Figure 12A**). To differentiate whether the effect emerges during gas administration or upon gas withdrawal, we then collected samples after 2 hours of continuous 50% N_2O administration and one hour after a 1-hour exposure. The upregulation of the examined IEGs was still evident in both groups (**Figure 12B**), indicating that excitatory activity takes hold during gas administration. Furthermore, we wanted to test whether shorter durations of gas exposure would be sufficient to upregulate excitatory markers. Indeed, phosphorylation of MAPK and expression of *c-fos* were upregulated after only a 30-minute exposure to N_2O (**Figure 12C**).

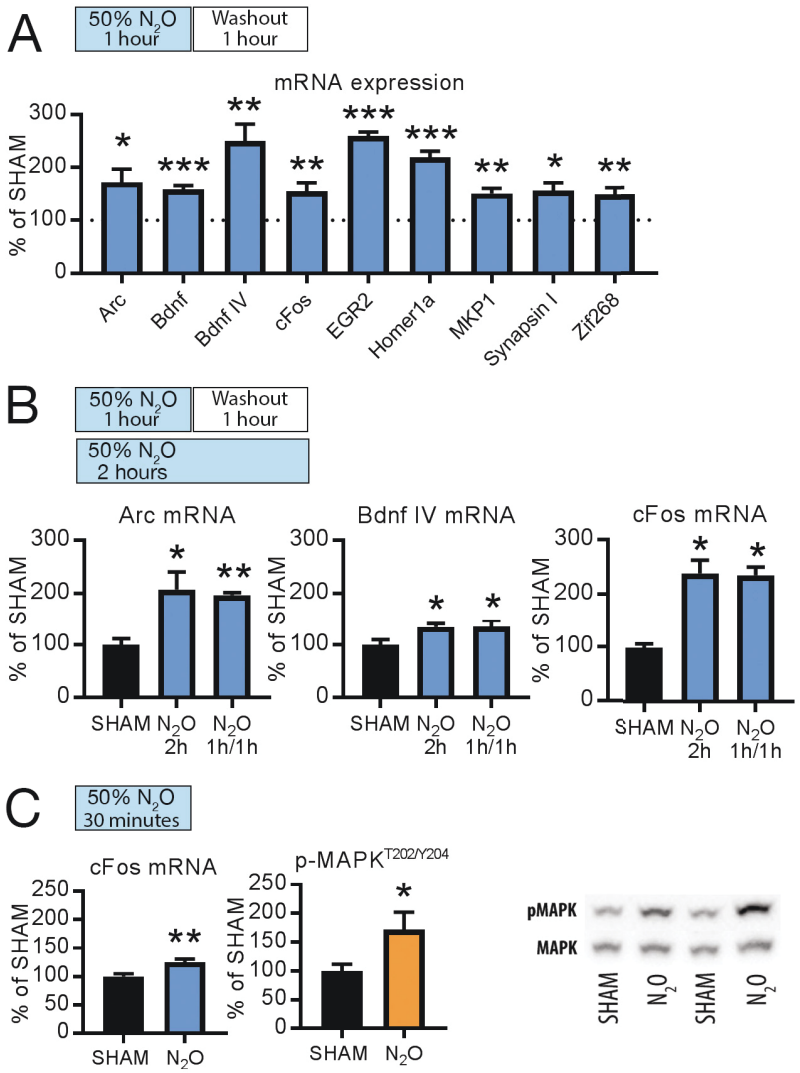

Figure 12. Nitrous oxide upregulates activity-dependent immediate-early genes. (**A**) Expression of *Arc*, *Bdnf*, *c-fos*, *Egr-2*, *Homer1a*, *Mkp1*, *Synapsin I*, and *Zif268* mRNA after 1-hour exposure to 50% N_2O and 1-hour washout in mouse PFC. (**B**) *Arc*, *Bdnf*, and *c-fos* mRNA are similarly upregulated after a 2-hour continuous N_2O administration (50% in O_2) and after a 1-hour washout following a 1-hour administration in mouse PFC. (**C**) A 30-minute continuous administration of N_2O (50% in O_2) upregulates *c-fos* mRNA and MAPK phosphorylation in mouse PFC. Data are presented as mean ± SEM. *< 0.05, **< 0.01, ***< 0.001, Student's/Welch's t-test (**A, C**) and Kurskal-Wallis one-way ANOVA followed by Dunn's *post hoc* test/one-way ANOVA, followed by Dunnett's *post hoc* test (**B**). Figure reprinted and modified from publication III.

To test whether N_2O activates TrkB signaling, we collected cortical samples immediately after a 30-minute administration of 50% N_2O. We assayed phosphorylation of TrkB, GSK3β, and p70S6K via Western blotting but observed no regulation, indicating that ongoing NMDAR blockade is not directly associated with activation of TrkB signaling (**Figure 13A**). However, in samples that were collected after N_2O exposure, during rebound SWA, TrkB signaling was significantly increased in all used N_2O concentrations (50%, 65%, 75%) (**Figure 13B**). Since 65% N_2O caused the largest increase in TrkB signaling, we used that concentration to test whether the signaling events are maintained at further time points. Indeed, the signaling was still upregulated 15 minutes after gas cessation (**Figure 13C**).

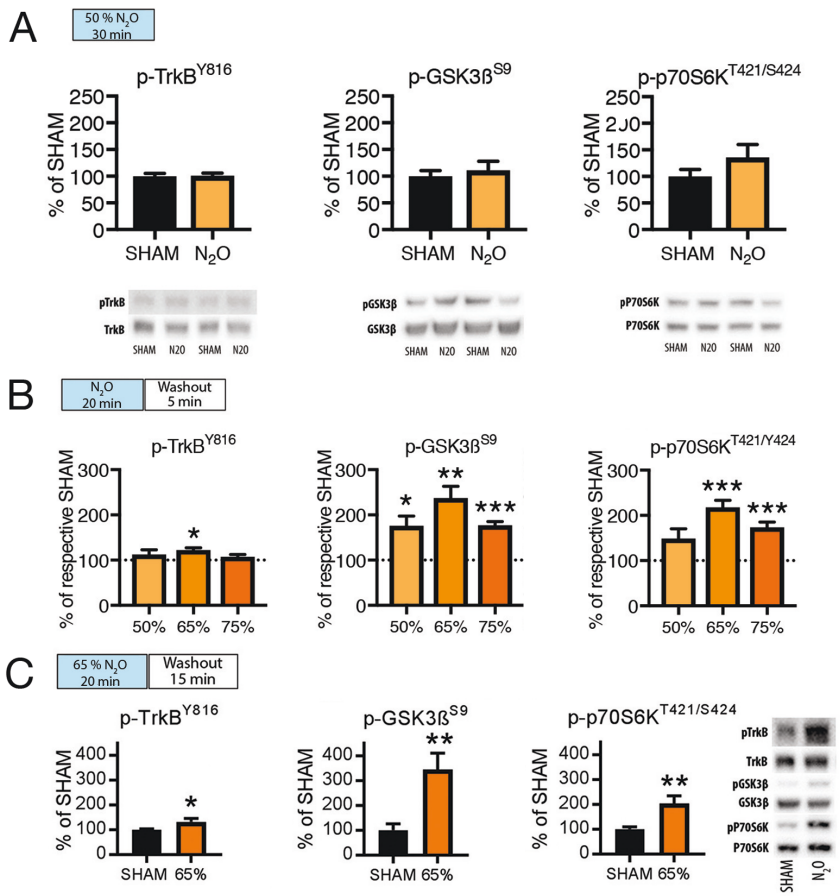

Figure 13. Dose- and time-dependent TrkB signaling effects of nitrous oxide. (**A**) Phosphorylation of TrkB, GSK3β, and p70S6K in mouse PFC remains unaltered during acute effects of 50% N_2O. (**B**) TrkB signaling is upregulated after a 5-minute washout from different N_2O concentrations. (**C**) Upregulation of TrkB signaling is maintained 15 minutes after treatment discontinuation of 65% N_2O. Data are presented as mean ± SEM. *< 0.05, **< 0.01, ***< 0.001, Student's/Welch's t-test (**A,B**) and Mann-Whitney test (**C**). Figure reprinted and modified from publication III.

To model ECT in rodents, we administered a volatile convulsant flurothyl on a cotton pad attached to the lid of an airtight Plexiglas chamber (10%, 100 µl/min) until the animals expressed generalized seizure behavior. The seizure was terminated by removing the chamber lid and attached cotton pad. A rapid emergence of SWA was observed after a chemically induced seizure by flurothyl (**Figure 14A**). TrkB signaling was robustly upregulated 10 minutes after the seizure (**Figure 14B**).

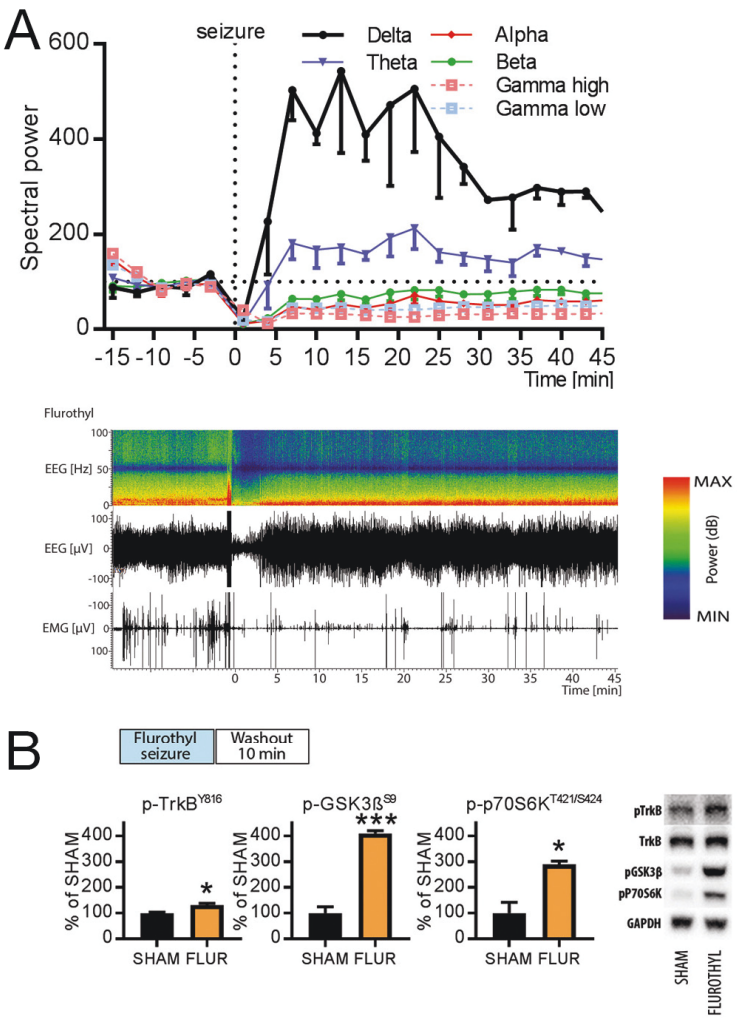

Figure 14. Flurothyl-induced seizure is followed by rebound SWA and TrkB signaling. (**A**) Normalized EEG power of main oscillations and representative time-frequency spectrogram after flurothyl-induced seizure. (**B**) TrkB signaling is upregulated during SWA 10 minutes after seizure. Data are presented as mean ± SEM. *< 0.05, ***< 0.001, Student's t-test. Figure reprinted and modified from publication III.

Next, we wanted to test whether a more direct pharmacological induction of SWA would also upregulate TrkB signaling. A systemic administration of sedative α2-adrenergic agonist medetomidine (0.05–0.3 mg/kg, i.p.) had no effect on activity-dependent IEG expression but robustly increased EEG SWA and phosphorylated TrkB, GSK3β, and p70S6K in PFC 30 minutes after administration (**Figure 15A–B**).

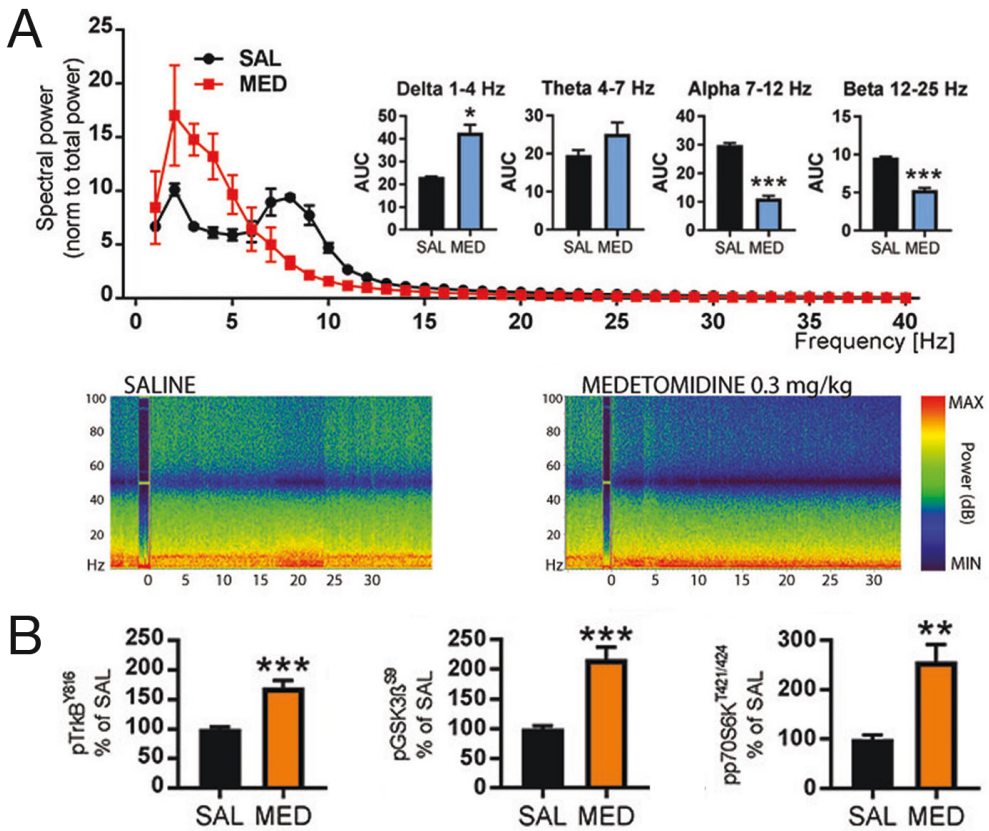

Figure 15. Medetomidine induces EEG SWA and TrkB signaling. (**A**) Spectral EEG power, major EEG oscillation frequency band power, represented as area under curve (AUC), and representative time-frequency spectrograms after 30 minutes of saline (SAL, i.p.) or medetomidine (MED, 0.3 mg/kg, i.p.) injections. (**B**) Medetomidine (0.05 mg/kg, i.p.) increases phosphorylation of TrkB, GSK3β, and p70S6K 30 minutes after treatment in mouse PFC. Data are presented as mean ± SEM. *< 0.05, **< 0.01, ***< 0.001, Student's/Welch's t-test. Figure reprinted and modified from publication III.

To see whether medetomidine's robust regulation of TrkB signaling would be reflected in an antidepressant-like effect similar to that produced by ketamine, we subjected mice to the learned helplessness model of depression. The animals that expressed the helpless phenotype after repeated exposures to foot shocks were administered saline, ketamine (10 mg/kg, i.p.), or medetomidine (0.05 mg/kg, i.p.), and the changes in helpless behavior were assessed 24 hours after treatment administrations. Unlike ketamine, medetomidine elicited no antidepressant-like response, as measured in failures to escape foot shocks, indicating that induction of TrkB signaling *per se* is not sufficient to elicit a rapid antidepressant-like behavioral response (**Figure 16**).

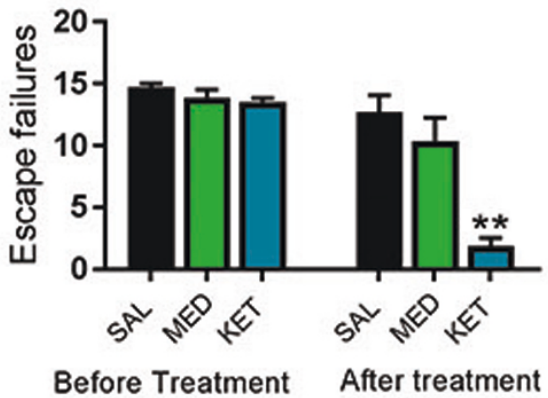

Figure 16. Ketamine—but not medetomidine—alleviates learned helplessness behavior in mice. Number of escape failures in a learned helplessness trial before and 24 hours after saline (SAL, i.p.), ketamine (KET, 10 mg/kg, i.p.), and medetomidine (MED, 0.05 mg/kg, i.p.) administrations. Ketamine caused a significant reduction in the number of escape failures, whereas medetomidine did not elicit similar antidepressant-like effects despite its ability to induce TrkB signaling. Data are presented as mean ± SEM. **< 0.01, Kruskal-Wallis one-way ANOVA. Figure reprinted and modified from publication III.

5.4. Ketamine dose-dependently increased TrkB signaling, independently of its HNK metabolites (IV)

Ketamine elicits its antidepressant-like effects particularly in subanesthetic doses, whereas higher anesthetic doses are considered inefficient (Li et al., 2010). However, in light of our finding of the sedative drug medetomidine's ability to elicit TrkB signaling, we were interested whether ketamine's ability to induce similar signaling effects would vary dose-dependently. In addition, a recent study proposed a metabolite of ketamine, HNK, to be responsible for ketamine's antidepressant effects, raising the question of whether HNK would also be responsible for ketamine-induced signaling effects (Zanos et al., 2016). In the fourth study, we investigated the dose-dependent effects of ketamine on EEG and TrkB signaling and determined whether the signaling effects are regulated by the HNK metabolite.

First, we tested the differential effects of subanesthetic (10 mg/kg, i.p.) and anesthetic (100 mg/kg, i.p.) doses of ketamine on mouse EEG. Subanesthetic ketamine caused a rapid increase in high frequency gamma oscillations (60–100 Hz) while lacking any major effects on other frequency bands during the 30-minute recording period (**Figure 17**). The anesthetic dose rapidly induced delta activity (1–4 Hz) while also modestly increasing theta (4–7 Hz), beta (12–25 Hz), and gamma oscillations (low: 25–40 Hz; high: 60–100 Hz) and decreasing alpha (7–12 Hz) oscillations.

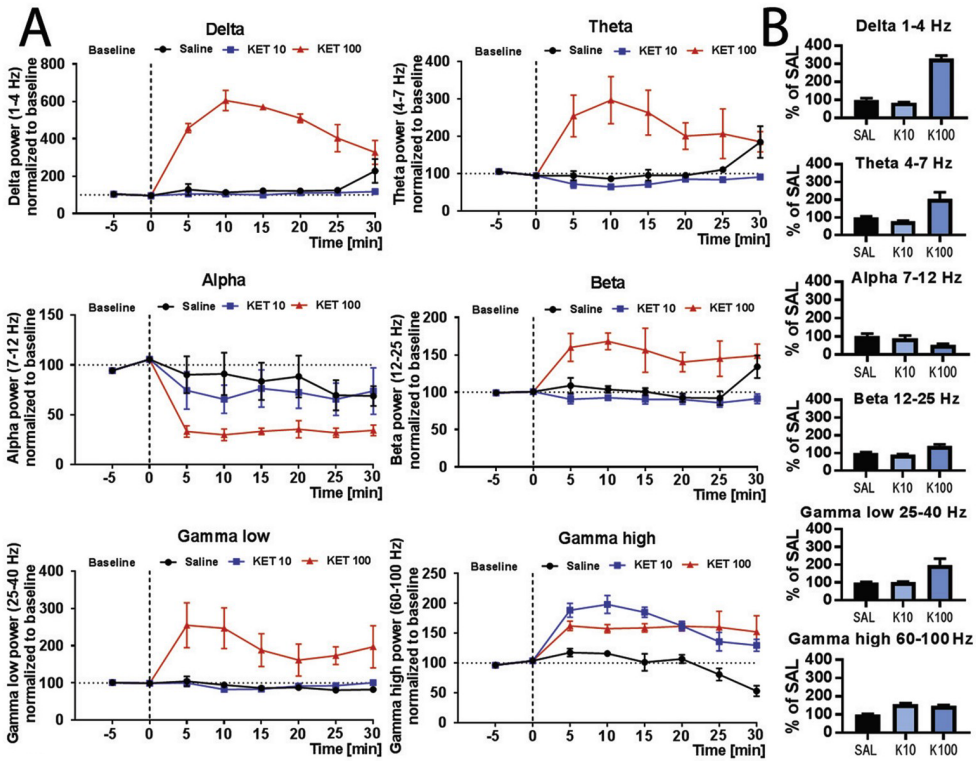

Figure 17. Effects of subanesthetic (10 mg/kg, i.p.) and anesthetic (100 mg/kg, i.p.) doses of ketamine on mouse EEG. (**A**) Normalized power of main EEG oscillations 30 minutes after ketamine administration (i.p.). (**B**) Major EEG oscillation frequencies as area under curve (AUC) representations for a 30-minute duration after treatment administration. Figure reprinted and modified from publication IV.

Next, we tested the effects of ketamine and HNK on TrkB signaling. An anesthetic (100 mg/kg, i.p.)—but not subanesthetic (10 mg/kg, i.p.)—dose of ketamine upregulated phosphorylation of TrkB, GSK3β, and p70S6K in mouse PFC 30 minutes after administration (**Figure 18A**), indicating that ketamine regulates TrkB signaling dose-dependently. This finding was supported by our findings of a dose-dependent regulation of TrkB signaling with two additional doses of ketamine (50 mg/kg and 200 mg/kg, i.p.) (**Figure 18B**).

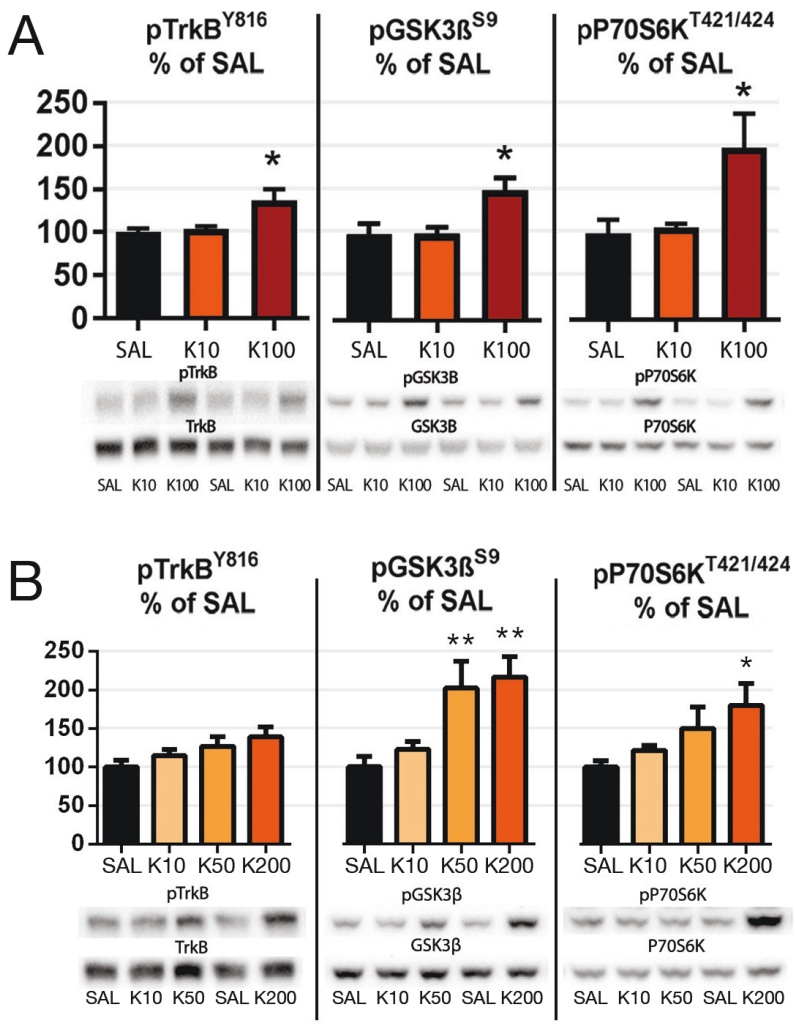

Figure 18. Ketamine dose-dependently upregulates TrkB signaling in mouse PFC. (**A, B**) Ketamine dose-dependently upregulates phosphorylation of TrkB, GSK3β, and p70S6K. Medial PFC samples were collected 30 minutes after i.p. injections. Data are presented as mean ± SEM. *< 0.05, **< 0.01, Kruskal-Wallis one-way ANOVA followed by Dunn's *post hoc* test (**A**) and one-way ANOVA followed by Dunnett's *post hoc* test (**B**). Figure reprinted and modified from publication IV.

To further examine the relevance of ketamine metabolism to its effect on TrkB signaling, we tested the effects of systemic *cis*-HNK administration (20 mg/kg, i.p.) on TrkB signaling. Furthermore, we synthesized a deuterated form of ketamine (6,6-dideuteroketamine). Ketamine deuterated at the C6 position has a decreased rate of metabolism to HNK. No signaling effects were observed 30 minutes after *cis*-HNK administration (**Figure 19A**), whereas deuterated ketamine (100 mg/kg, i.p.) upregulated phosphorylation of TrkB, GSK3β, and p70S6K in mouse PFC 30 minutes after administration similarly to ketamine (**Figure 19B**), indicating that ketamine's ability to induce TrkB signaling is independent of its metabolism to HNK.

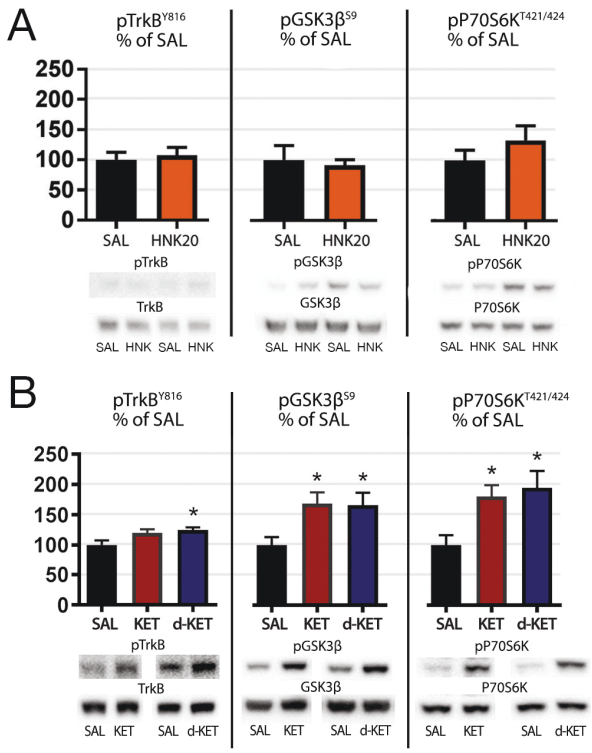

Figure 19. (**A**) Cis-6-hydroxynorketamine (HNK, 20 mg/kg, i.p.) caused negligible effects on TrkB signaling. (**B**) Deuteroketamine (d-KET, 100 mg/kg, i.p.) regulates phosphorylation of TrkB, GSK3β, and p70S6K similarly to ketamine (100 mg/kg, i.p.). Mouse PFC samples were collected 30 minutes after administration. Data are presented as mean ± SEM. *< 0.05 One-way ANOVA followed by Dunnett's *post hoc* test. Figure reprinted and modified from publication IV.

Overall, our findings show that ketamine preferentially regulates TrkB signaling in higher, anesthetic doses and that the signaling effects are not dependent on ketamine's metabolism to HNK. In addition, our findings show that ketamine-induced TrkB signaling is associated with increased EEG SWA.

6. Discussion

Anesthetics are strong modulators of excitatory and inhibitory neurotransmission. While mainly used in surgical operations, anesthetics have relatively recently gained wider interest in their potential in the treatment of mood disorders. Subanesthetic ketamine, in particular, has demonstrated remarkably rapid antidepressant features, but other anesthetic agents, such as isoflurane, N_2O, and propofol have shown promise as well (Berman et al., 2000; Langer et al., 1995; Mickey et al., 2018; Nagele et al., 2015; Weeks et al., 2013). Despite this, accumulating knowledge of anesthetic function has raised serious concerns over the drugs' potentially detrimental effects on the developing brain (Jevtovic-Todorovic et al., 2003). In the present thesis, we have investigated various aspects of anesthetic function, from the drugs' potential long-lasting adverse effects to their underlying antidepressant mechanisms.

6.1. Developmental stage-dependent effects of early postnatal anesthesia

Anesthetics have been consistently shown to have differential effects on the developing nervous system in rodents, depending on the specific stage of CNS maturation during anesthesia exposure (Briner et al., 2011; Qiu et al., 2016). Repeated exposure to anesthetics during early postnatal development has been associated with learning deficits later in life (Flick et al., 2011; Wilder et al., 2009). These findings are supported by experimental evidence in rodents demonstrating that early postnatal anesthesia increases apoptotic neurodegeneration (Jevtovic-Todorovic et al., 2003; Tagawa et al., 2014). In contrast, anesthesia during the third postnatal week in rodents has been shown to increase dendritic spine density (Briner et al., 2010; De Roo et al., 2009). However, the long-term physiological significance of these alterations, and the relevance for the depth and duration of anesthesia remain poorly elucidated. Deleterious effects of early-life anesthesia would have significant clinical implications due to the widespread use of anesthetics for surgical anesthesia, mild sedation, and analgesia in pediatric medicine. Furthermore, along with the continuous advances in modern preclinical invasive techniques (e.g., *in vivo* imaging), the use of anesthetics is steadily growing in veterinary medicine and biomedical research involving animals, and any permanent effects that anesthesia may have beyond its acute effects pose a significant confounding factor in the interpretation of these experimental findings. In light of the differential effects of early-life anesthesia, we hypothesized that repeated isoflurane anesthesia administered at different developmental time points would have differing effects on adult behavior.

In the first study of the present thesis, a 30-minute isoflurane anesthesia on three consecutive days at two distinct time points during early development caused modest effects in adult behavior. These behavioral alterations, observed in overall locomotor activity, spatial learning, and memory, were dependent on the time points of exposure to anesthesia or maternal separation. Our findings are in line with a recent study that demonstrated negligible long-

lasting behavioral effects of a brief early-life anesthesia with sevoflurane (Qiu et al., 2016). Duration of anesthesia may play a significant role since a 6-hour anesthesia has been shown to have detrimental long-lasting consequences (Briner et al., 2011). Further, repeated sevoflurane anesthesia (3 x 2 hours) caused more significant synapse loss than a single 6-hour anesthesia (Amrock et al., 2015). Therefore, it is plausible to speculate that a longer exposure to isoflurane anesthesia may produce more noticeable behavioral effects than those observed in our study.

A rodent brain goes through rapid and fundamental changes between the first and second postnatal weeks—during the brain growth spurt and critical period—which may help in explaining the differential effects of anesthetics at different developmental time points (Clancy et al., 2001). For instance, the function of GABAergic neurotransmission, a major target of anesthetic function, changes from depolarizing to hyperpolarizing due to developmental changes in intracellular Cl^- concentration, brought about by gradual upregulation of neuronal potassium-chloride cotransporter KCC2 expression during brain maturation (Rivera et al., 1999).

One limitation of the study is that no measurements of apoptotic markers or dendritic spine density were made following our anesthesia regimen, therefore limiting the conclusions that can be drawn from our data. Our study focuses on general behavioral phenomena in canonical behavioral tests, but the molecular events associated with the potential long-term deleterious effects of isoflurane remain to be elucidated. Furthermore, despite our use of a wide behavioral battery, some subtler deficits not addressed in our test battery may still remain unnoticed. Translational extrapolation of our results to humans are limited due to major differences in the progress of brain development between species and the limited applicability of the behavioral phenotype of rodents to humans (Clancy et al., 2001). None of the selected behavioral tests can fully recapitulate human diseases but may reveal phenotypical characteristics that resemble certain pathological states (e.g., the prepulse inhibition test for schizophrenia).

As of now, conflicting evidence exists regarding the long-term harms of early-life anesthesia (Yu et al., 2013). Anesthetics provide the means to perform surgical operations whose benefits may far outweigh the potential deleterious effects of anesthesia, but currently available data advises caution and consideration, especially for repeated anesthesia exposures during childhood and adolescence. The exposure duration likely plays a significant role in the potential detrimental effects of anesthesia since a recent clinical trial demonstrated that just under an hour of general anesthesia during early infancy caused no notable neurodevelopmental disturbances (McCann et al., 2019).

6.2. Negligible effects of isoflurane anesthesia in the rat CMS model

In study II, we tested the antidepressant-like effects of repeated isoflurane anesthesia on a rat CMS model. Isoflurane has been shown to alleviate depressive symptoms in preliminary clinical trials, but many subsequent findings have remained contradictory (Greenberg et al., 1987; Langer et al., 1985, 1995). Recent clinical and preclinical studies demonstrating the antidepressant potential of isoflurane have renewed interest in the study of isoflurane in an antidepressant context (Antila et al., 2017; Brown et al., 2018a; Weeks et al., 2013). However, in our experiment, repeated isoflurane anesthesia failed to alleviate anhedonic behavior in rats exposed to CMS. This lack of effect was accompanied by—and potentially connected to—a lack of regulation of cortical and hippocampal BDNF expression. Our findings therefore provide no direct support for the antidepressant potential of isoflurane anesthesia per se. However, the optimal dose and duration of isoflurane "narcotherapy," as coined by Langer and colleagues (1995), remain largely unknown. Preclinical and clinical studies have demonstrated an antidepressant-like efficacy of isoflurane with various administration protocols (Antila et al., 2017; Brown et al., 2018a; Langer et al., 1995; Weeks et al., 2013). In a recent study, Zhang and colleagues (2019) demonstrated that only a single isoflurane anesthesia for 30 minutes (4–3% induction, 2% maintenance) renormalized behavioral deficits induced by CMS in mice. BDNF expression in the PFC was also shown to be decreased after CMS and renormalized to basal levels after isoflurane administration, indicating that the antidepressant potential of isoflurane may be connected to an administration routine that induces BDNF upregulation. Our findings conflict with these observations, which may be due to our use of a highly stress-sensitive substrain of rats that are resistant to citalopram after CMS exposure (Neyazi et al., 2018). Unaltered BDNF levels after isoflurane anesthesia, however, have also been reported, indicating a degree of unpredictability in isoflurane's capability to influence BDNF expression (Antila et al., 2017).

It is also debatable whether the CMS model is a sufficient method of modeling clinical depression in rodents. Although the validity of the CMS model is relatively strong as the behavioral phenotype includes many aspects reminiscent of a depressive state, including anhedonia, sleep pattern disturbances, weight loss, and decrease in motivated behaviors, some discrepancies remain (Willner, 2017). The depression-like symptoms, namely anhedonia, as measured by sucrose consumption, quickly return to basal level once the mild stressors are stopped, whereas major depression in humans is usually more persistent (Grippo et al., 2003). In addition, the CMS model has raised controversy due to its poor reproducibility, which was evident in our study as well since only 53.8% of the stress-exposed rats developed the anhedonic phenotype (Krishnan & Nestler, 2011). Similar shortcomings in symptom persistency and reproducibility are also associated with the learned helplessness model used in study III. Nevertheless, depressive-like behaviors, such as anhedonia, behavioral despair, and helplessness, in rodents can be consistently renormalized with antidepressants (Willner,

2017). However, these treatment responses are mainly observed with conventional monoaminergic antidepressants, and their responsiveness to novel non-monoaminergic drugs remains to be clarified.

6.3. Increased TrkB signaling during rebound SWA—a primer for a shared mechanism of rapid-acting antidepressants?

In studies III and IV, we aimed to gain more insight into the neurobiological mechanisms underlying rapid antidepressant effects by examining the EEG and molecular effects of antidepressant anesthetics, such as N_2O and ketamine. N_2O has been shown to alleviate depressive symptoms in a small-scale clinical trial, but the molecular mechanisms related to these effects are still rarely studied (Nagele et al., 2015). N_2O would have several clinical benefits over ketamine if its antidepressant potential proves plausible. The administration of N_2O can be easily controlled due to its fast pharmacokinetics and negligible metabolism. The acute effects of N_2O emerge within seconds of administration and cease within minutes of treatment discontinuation (Nagele et al., 2018). However, the misuse potential and adverse effects of N_2O must be taken into consideration (van Amsterdam et al., 2015). Heavy and repeated use of N_2O has also been associated with vitamin B_{12} deficiency.

By taking advantage of the rapid pharmacokinetic properties of N_2O, we discovered that N_2O induces an emergence of EEG SWA that is coupled with upregulation of TrkB signaling in mouse PFC. This effect emerges only after drug discontinuation. The acute effects of N_2O, in contrast, were characterized by the upregulation of activity-dependent IEGs, but effects on TrkB signaling remained negligible. A similar phenomenon of rebound SWA, coupled with increased TrkB signaling, were observed after seizures chemically induced by a volatile convulsant, flurothyl. However, direct induction of SWA by the sedative drug medetomidine proved ineffective in producing an antidepressant-like response in the learned helplessness model even though it readily upregulated TrkB signaling.

Furthermore, in study IV, we also demonstrated that ketamine induces TrkB signaling during SWA. These effects were not limited to subanesthetic doses but rather were more pronounced with higher, anesthetic doses. We also found these signaling effects to be independent of ketamine's metabolism to HNK. However, the conclusions that can be drawn on the ability of HNK to initiate TrkB signaling based on our current study are limited. In our study, we examined the signaling effects of HNK only in a single dose and time point post-administration, and focused on changes in the PFC. Our study does not, therefore, rule out the possibility that HNK might elicit signaling responses similar to ketamine in other doses, time points, or brain regions.

Overall, these findings indicate that TrkB signaling, although likely a crucial component of rapid antidepressant responses, as indicated by a lack of antidepressant effects of ketamine in

conditional *Bdnf*$^{-/-}$ rodents (Autry et al., 2011), is not in itself sufficient to elicit a rapid antidepressant response. Instead, N_2O, flurothyl, and subanesthetic ketamine share the capability to induce cortical excitation before the effects on TrkB signaling emerge. Based on these findings, we have formulated a hypothesis of the two phases of rapid antidepressant action (Kohtala, 2019) (**Figure 20A**). The hypothesis proposes that for a drug to function as a rapid-acting antidepressant, it must elicit an initial phase of transient cortical excitation that can be characterized, for instance, by upregulation of activity-dependent IEGs or MAPK phosphorylation, or an increase in EEG gamma oscillations. This excitatory phase is followed by a subsequent sedative-like state, during which SWA emerges alongside neurotrophic signaling.

In regular physiological conditions, BDNF is expressed as a response to neuronal activity (Park & Poo, 2013; Thoenen, 1995). Global cortical excitation is evident during seizure manifestation in ECT and is correspondingly shown to increase BDNF expression (Altar et al., 2003, 2004; Angelucci et al., 2002; Nibuya et al., 1995), but the importance of postictal EEG silencing in ECT's therapeutic effect has also been emphasized (Nobler et al., 1993; Sackeim et al., 1996; Suppes et al., 1996). Isoflurane shares the capability to produce electrocerebral silence with ECT but without the preceding seizure activity (Langer et al., 1995). However, excitatory responses have been reported in association with isoflurane anesthesia, particularly during anesthesia induction and emergence, when the concentration of the anesthetic is low (Voss et al., 2008). In rodents, isoflurane produced hyperactivity during anesthesia induction, while in rodent brain slices, low isoflurane concentrations enhanced neuronal excitability (Becker et al., 2012; Ou et al., 2020). In clinical practice, a state of restless, agitated, and incoherent behavior is well-recognized during the induction and emergence of anesthesia with several general anesthetics, including isoflurane (Costi et al., 2014; Moore & Anghelescu, 2017). Interestingly, an increased plasma BDNF level has been shown to correlate with emergence agitation in elderly patients after propofol-induced anesthesia (Mei & Tong, 2016). In addition to anesthesia induction and emergence, cortical hyperexcitability has been reported during deep burst-suppressing anesthesia (Ferron et al., 2009; Kroeger & Amzica, 2007). Therefore, it is tempting to speculate that an administration regimen capable of producing cortical excitation and an accompanying BDNF upregulation may provide a more robust antidepressant-like effect. The present hypothesis would account for the lack of antidepressant-like effects of isoflurane anesthesia since it may not consistently induce similar biphasic actions (**Figure 20B**). Further studies to clarify the antidepressant potential of isoflurane in different dosing regimens are therefore warranted.

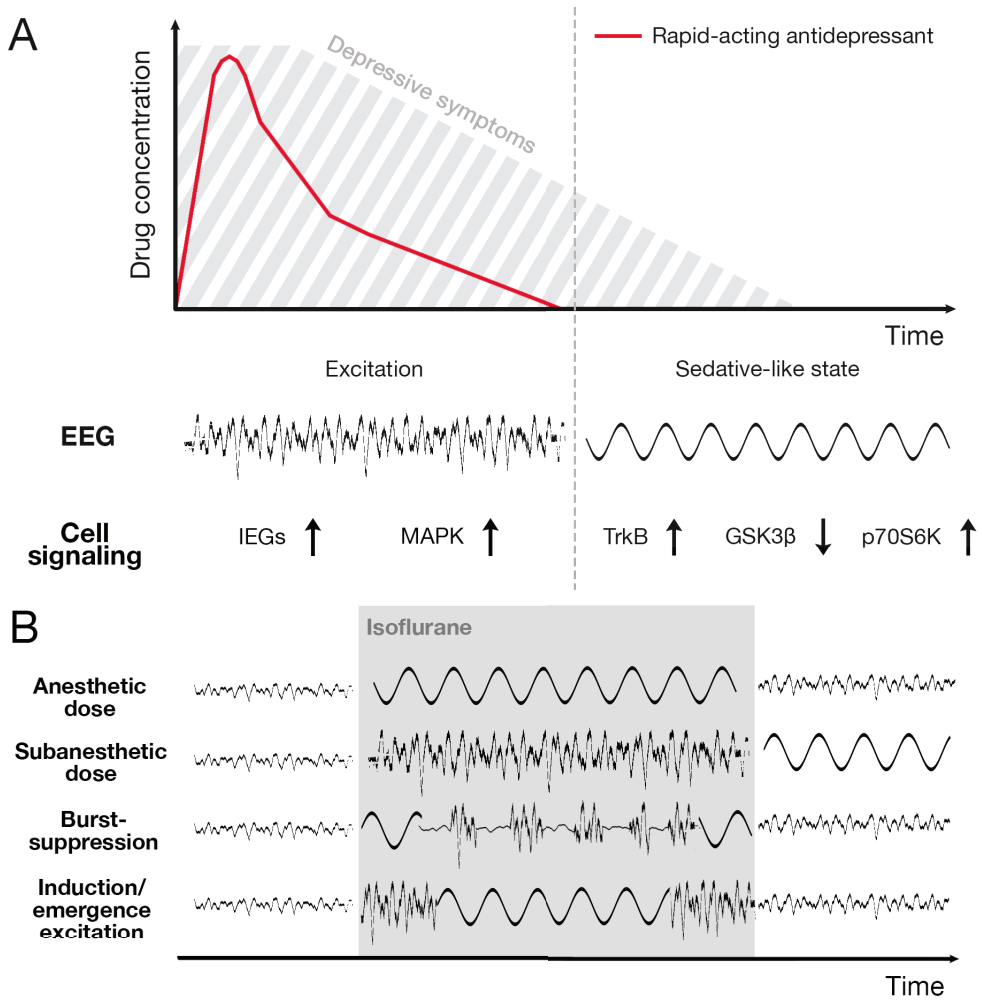

Figure 20. Hypothesis of biphasic effects of rapid-acting antidepressants. (**A**) Our findings suggest that rapid-acting antidepressants share the capability to induce cortical excitation, as exemplified by upregulation of activity-dependent immediate-early genes during the acute drug effects (left panel), and to induce slow-wave EEG activity accompanied by upregulated TrkB signaling after the peak pharmacological effects subside (right panel). (**B**) According to the hypothesis, isoflurane may lack antidepressant-like effects in dosing that produces insufficient excitatory response. However, isoflurane may exert paradoxical cortical excitation at low subanesthetic dosing, during burst-suppression, and during anesthesia induction and emergence.

The functional significance of the biphasic actions observed with N_2O, subanesthetic ketamine, and flurothyl remains to be elucidated. Interesting hints may, however, be offered by the physiology of sleep since the EEG SWA pattern that we observed after treatment discontinuation is also characteristic of deep non-rapid eye movement (NREM) sleep. A prominent hypothesis of sleep, the synaptic homeostasis hypothesis (SHY), postulates that NREM sleep SWA increases as a function of preceding synaptic potentiation during wakefulness and represents a phase of downscaling of synaptic strength (Tononi & Cirelli, 2003). This downscaling allows for the maintenance of synaptic homeostasis so that saturation of synaptic connections is avoided and headroom for synaptic plasticity and learning is maintained. It is therefore tempting to speculate that similar homeostatic mechanisms may apply to rapid-acting antidepressant function as well. Indeed, ketamine has been shown to increase SWA in the night following the administration in both humans and rodents (Duncan et al., 2013; Feinberg & Campbell, 1993). The increase in ketamine-induced sleep SWA was also reported to be proportional to an increase in plasma BDNF levels in treatment responders (Duncan et al., 2013). Intriguing similarities have also been observed with sleep deprivation, a somatic treatment for depression that has been shown to induce cortical excitability during prolonged wakefulness, followed by increased SWA during the recovery sleep period (Campbell & Feinberg, 1999; Huber et al., 2000, 2013). Since we found SWA and TrkB signaling to emerge only after the acute pharmacological effects of the treatments subside, it is plausible to hypothesize that these phenomena are an inherent response of the brain to an excitatory challenge, regardless of whether it is mediated by an excitatory drug such as N_2O or ketamine, a generalized seizure induced by ECT or flurothyl, or through prolonged wakefulness.

Indeed, a recent proposal suggests the initial phase of cortical excitation during the acute effects of rapid-acting antidepressants represents a phase of increased synaptic potentiation through molecular mechanisms reminiscent of LTP, including rapid transcription of plasticity-related IEGs, such as *Arc* and *Bdnf* (Rantamäki & Kohtala, 2020). These changes ultimately lead to acute changes in the functional connectivity of neural networks, but the effects would remain transient without further consolidation. In accordance with the SHY hypothesis, the homeostatic emergence of SWA and accompanying molecular signaling mechanisms following the acute effects of rapid-acting antidepressants may reflect a subacute consolidation of synaptic changes induced by the acute excitatory effects of the investigated anesthetics, and ultimately contribute to the reconfiguration of functional network connectivity, which is pathologically altered in depression (Rantamäki & Kohtala, 2020). Essentially, our findings suggest that reproducing this biphasic phenomenon may be more important than the treatment's engagement of an acute pharmacological target in mediating rapid antidepressant responses. This is further supported by the mechanistic diversity of rapid-acting antidepressant treatments, which range from the NMDAR antagonism of ketamine and N_2O to non-pharmacological treatments, such as ECT and sleep deprivation, and potentially to the serotonergic agonism of classical psychedelics.

The findings linking rebound EEG SWA and increased TrkB signaling in studies III and IV remain largely correlational. However, the present hypothesis provides a novel framework for additional clinical and preclinical studies that encourages widening the perspective of mechanistic antidepressant studies from the acute pharmacology of the given treatment to the adaptive changes and homeostatic responses of the brain to a drug challenge. The importance of further studies on the mechanisms of rapid-acting antidepressants is emphasized by the recent approval of intranasal esketamine as an adjunct for oral antidepressants for treatment-resistant depression in adults in the US and Europe. This will likely increase clinical use of ketamine in the coming years. Our findings indicate that a subanesthetic bolus of ketamine may be sufficient to reproduce the biphasic effect observed in our studies, but this effect needs to be confirmed in human studies and in an intranasal administration route. The increasing clinical use of ketamine also calls for strategies to mitigate the risks of its adverse effects and misuse potential. Such strategies currently include special regulations and monitoring, such as administration of the treatment under clinical supervision.

7. Conclusions

In this thesis, I examined the behavioral and molecular effects of various anesthetics indicated to have rapid-acting antidepressant potential. The findings presented here demonstrate a shared phenomenon in EEG and TrkB signaling between pharmacologically diverse rapid-acting antidepressants.

The main conclusions are:

I Repeated exposure to brief but deep isoflurane anesthesia during postnatal days 7–9 or 15–17 causes minor effects on the general locomotor activity, spatial learning, and memory in adult male mice. The behavioral effects are dependent on the developmental time point of anesthesia exposures.

II Repeated exposure to brief but deep isoflurane anesthesia lacks antidepressant-like effects in the chronic mild stress model in adult rats. The lack of behavioral effects is consistent with unchanged levels of cortical and hippocampal BDNF expression.

III Nitrous oxide and subanesthetic ketamine induce EEG slow-wave activity after their peak pharmacological effects subside, resembling postictal silencing following electroconvulsive therapy or volatile convulsant flurothyl. The slow-wave activity is accompanied by upregulation of TrkB signaling.

IV Medetomidine does not produce antidepressant-like effects in the learned helplessness model of depression in mice despite readily upregulating slow-wave activity and TrkB signaling.

V Ketamine induces TrkB signaling dose-dependently. The effects are independent of its hydroxynorketamine metabolite and appear preferentially at high sedative-anesthetic doses.

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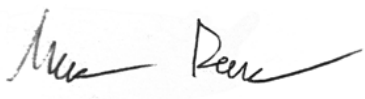

Marko Rosenholm

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